UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

RIGHT DORSAL COLON ULTRASONOGRAPHY IN HORSES

PATRÍCIA DA SILVA CALDEIRA

CONSTITUIÇÃO DO JÚRI

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Once upon a time...
A dream of a child, becoming an adult profession ...

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There are so many! I hope I will not forget anyone ...

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Resumo

Avaliação ultrassonográfica do Cólon Dorsal Direito em Equinos

A Colite Dorsal Direita é uma enteropatia inflamatória e ulcerativa frequente do cólon dorsal direito (CDD) em equinos, onde existe perda de proteínas através de uma parede intestinal comprometida, habitualmente associada a uma administração de anti-inflamatórios não esteroides (AINEs).

O objetivo deste estudo prospectivo foi o de avaliar a espessura da parede do CDD em cavalos saudáveis (grupo A) e sob o tratamento (grupo B) com AINEs, através da ecografia transabdominal, juntamente com valor das proteínas totais e albumina séricas, antes (T1) e depois (T2) do tratamento. Para cada animal foi registado a idade, género e raça, e o espaço intercostal (EIC) onde foi visualizado o CDD. A associação entre os parâmetros estudados foi investigada. Para isso foram realizadas ecografias a todos os cavalos militares do 4º Esquadrão da Guarda Nacional Republicana (GNR), durante 6 meses, que por razões médicas necessitaram de um tratamento com AINEs.

Todos os cavalos foram Puro Sangue Lusitanos (PSL) com uma média de idade de 11,02 ± 6,37 anos e de ambos os géneros. O grupo A é constituído por 26 cavalos, e o grupo B por 22 cavalos. A média de espessura da parede do CDD foi de 2,7 ± 0,76 mm, sendo os EIC de melhor visualização os 12º, 13º e 14º do lado direito. A idade foi o único parâmetro que influenciou positivamente a espessura da parede do CDD com p-value = 0,0247, em T1, indicando um aumento da espessura da parede do CDD com um aumento da idade do animal. Da regressão linear, obteve-se a seguinte equação: Y= 2,17037 + 0,03926X, onde X= idade, em anos, e Y= espessura média da parede do CDD, mm. O género, raça, PT, Alb, e tipo de AINE não influenciaram, estatisticamente, a espessura média da parede do CDD. A duração do tratamento com AINEs, em dias, demonstrou ser estatisticamente significativa (p-value = 0,049), por outras palavras, a espessura da parede do CDD aumenta com a duração do tratamento com AINEs. Concluiu-se que factores como a idade do animal e a duração do tratamento com AINEs aumenta a espessura da parede no cólon dorsal direito em cavalos PSL. Com base no conhecimento dos autores, este é o primeiro relato a identificar parâmetros como a idade do animal e a duração do tratamento com AINE, a causar um efeito significativo na espessura da parede do cólon dorsal direito em cavalos.

Palavras chave: Colite Dorsal Direita; AINEs; equino; ecografia abdominal; albumina; proteínas totais.

Abstract

Ultrasound evaluation of the Right Dorsal Colon in equines

Right Dorsal Colitis is a frequent ulcerative inflammatory and ulcerative disease of the right dorsal colon (RDC) in horses, accompanied by protein loss, due to a compromised intestinal wall, usually associated with the administration of non-steroidal anti-inflammatory drugs (NSAIDs).

The objective of this prospective study was to evaluate the wall thickness of the RDC in healthy horses (group A) and in horses under NSAIDs treatment (group B) through a transabdominal ultrasound, together with serum measurement of total protein and albumin before (T1) and after (T2) treatment. For each animal the age, gender, breed, and the identification of the intercostal space (ICS) were recorded. The association between the studied parameters was investigated. For this, ultrasound scans were performed on all military horses of the 4th Esquadrão of Guarda Nacional Republicana (GNR) for 6 months, which for medical reasons required a NSAID treatment.

All horses were Puro Sangue Lusitano (PSL) with a mean age of 11,02 ± 6,37 years and both genders. Group A included 26 horses, and group B, 22 horses. Overall, the average wall thickness of the RDC was 2,7 ± 0,76 mm, with the best visualization on 12th, 13th and 14th ICS right sided. Age was the only parameter with a significant positive influence on the RDC wall thickness, with p-value = 0,0247, at T1, indicating an increase in RDC wall thickness with increasing age of the animal. From linear regression, the following equation was obtained: Y = 2,17037 + 0,03926X, where X = age, in years, and Y = mean RDC wall thickness, mm. Gender, breed, PT, Alb, and type of NSAIDs was not statistically significant on the mean wall thickness of the RDC. The duration of NSAID treatment in days showed a statistically significant effect (p-value = 0,049), in other words, the wall thickness of the RDC increases with the duration of NSAID treatment. To the authors knowledge, this is the first report suggesting that the age of the animal and the duration of NSAID treatment have a significant effect on the wall thickness of the right dorsal colon, although others studies need to evaluate the effect of these parameters on others horse's breed.

Key words: Right Dorsal Colitis; NSAIDs; equine; abdominal ultrasound; albumin; total proteins.

Table of Contents

List of Abbreviations and Symbols

AA - Arachidonic Acid

AINEs – Anti-inflamatórios não esteroides

Alb – serum albumin

CDD – Colon dorsal direito

cm- centimeter

COX - Cyclooxygenase

COX-1 – Cyclooxygenase 1

COX-2 – Cyclooxygenase 2

COX-3- Cyclooxygenase 3

EIC- Espaço Intercostal

FM – Flunixin Meglumine

g/dl – grams per deciliter

GALT- Gut Associated Lymphoid Tissue

GI – Gastrointestinal

GNR – Guarda Nacional Republicana

ICS- Intercostal Space

IL-1 α – Interleukin 1α

IL-1β – Interleukin 1β

IL -15 – Interleukin-15

IL-18 – Interleukin 18

IFNγ – Interferon γ

Ketp - Ketoprofen

L- liters

LDC – Left Dorsal Colon

LO- lipoxygenase

LPS – Lipopolysaccharide

LVC- Left Ventral Colon

m - meter

mm – millimeter

N – Number of observations

NSAID – NonSteroidal Anti-Inflammatory Drug

PBZ - Phenylbutazone

PC – Patrícia Caldeira

PG - Prostaglandins

PGI2 – Prostacyclin

PSL– Puro Sangue Lusitano

PT - Total Proteins

RDC- Right Dorsal Colon

RDColitis- Right Dorsal Colitis

RF – Prof. Rita Fonseca

RVC- Right Ventral Colon

s.d. – standard deviation

TCD4 + cells – T helper cells

TGFβ1 – Transforming growth factor beta 1

TNFα – Tumour necrosis factor

USHE- Unidade de Segurança e Honras de Estado

VFAs – Volatile Fatty Acid

WBC – White Blood Cells

% - Percentage

List of Figures

List of Tables

List of Graphs

I. Internship Report

As part of the Integrated Master's Degree in Veterinary Medicine from the Faculty of Veterinary Medicine, University of Lisbon, I completed a 3-month training at the Equine Hospital of the 4º Esquadrão da Guarda Nacional Republicana (GNR) in Lisbon, from September to December of 2018. Followed by an internship in the Equine Hospital of Extremadura, Cáceres, in Spain.

A brief description of the activities developed during both internships is presented here.

Equine Hospital of the 4ºEsquadrão da Guarda Nacional Republicana (GNR) - Unidade de Segurança e Honras de Estado (USHE) is located in Calçada da Ajuda, in Lisbon, and offers medical-veterinary services to the horses belonging to the national staff of GNR. The clinical staff includes four captain veterinarians, two veterinary nurses and two auxiliaries with training aimed at clinical practice. The hospital is equipped with an administrative office, a pharmacy, a treatment room with stalls, which communicates with the induction/ recovery room, and the later with the surgical theatre. The hospital has the capacity to keep twelve horses in the intensive care unit. There is a sterilization room for surgical equipment. Other facilities include a mechanical walker and different riding arenas. The hospital is equipped with material for radiologic and ultrasonographic diagnosis.

Figure 1- Mechanical walker

Figure 2- Boxes

Figure 3- Reception space.

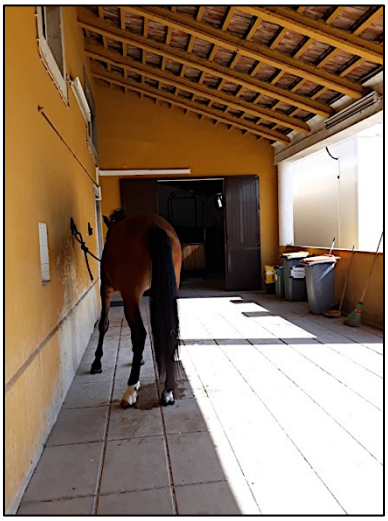


Figure 4- Treatment room.

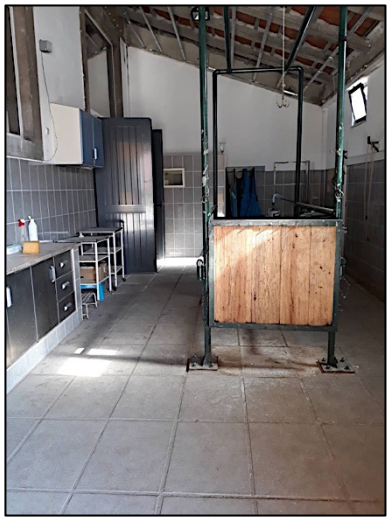


Figure 5- Intense Unit Care.

From the 17th of September to the 21st of December of 2018, I took a dynamic participation on the daily clinical work of the hospital; I also developed the experimental study of my master thesis, on the area of "Equine Right Dorsal Colitis". Indeed, the elaboration of this experimental protocol was performed in the GNR hospital facilities from September 17st of 2018 to May 31st of 2019. Both supervisors, Prof. Rita Fonseca Pequito and Dr. Daniela Teixeira supervised this project.

Regarding my practical internship in the GNR hospital, Dr. Daniela Teixeira was my supervisor during the internship. A typical day's work at the hospital was characterized by morning routine treatments of the hospitalized horses, which I actively participated in, while during the afternoon I accompanied the veterinarians during consultations. The activities carried out during this internship period were:

- General physical examination;
- Preparation and administration of all oral, topical and intramuscular/intravenous treatment
- Active participation in the process of horse shoeing, and in discussions with the farriers and veterinarians about the best shoeing option for each specific case.
- Deworming and vaccination;
- Cleaning of wounds and bandages
- Rectal palpation for colic diagnosis;
- Nasogastric intubation;

- Lameness examination;
- Perineural and intraarticular blocks;
- Radiographic and ultrasound examination;
- Leveling of the dental arcade;
- Mesotherapy;
- Unblocking the tear duct;
- Orchiectomies: standing and decubitus approaches;
- Active participation in surgeries and general anesthesia;
- Active participation as part of the medical team in equine competitions;
- Euthanasia.

The hospital of the 4º Esquadrão of GNR lodges nearly 200 horses, whose functions vary between daily patrols, courses of Public Order and Equitation, Riding School, Reprise presentations, competitions in show jumping, dressage and 3day-eventing. Most lesions were related with the musculoskeletal apparatus (such arthritis, tendinitis, laminitis, rhabdomyolysis, hoof abscesses and various wounds). However, in the gastrointestinal tract, colic due to impaction of the pelvic flexure and gas distension of the cecum were the most frequent diagnoses with subsequent medical resolution. In addition, dentistry work, included several leveling of the dental arcade, being one of the most common riders' complaints.

II. Literature review

Introduction

In equine medicine, the prescription of non-steroidal anti-inflammatory drugs (NSAIDs) is common practice (Lees & Higgins, 1985; Tomlinson & Blikslager, 2003; Soma, Uboh & Maylin, 2011) and until 1979 it was believed that 100% of the effect was beneficial, mainly broken down by analgesic and anti-inflammatory properties (Tobin *et al*., 1986). Both NSAIDs act in blocking the production of physiologically important prostaglandins in the homeostasis of the organism and in blocking the production of prostaglandins induced by damaging stimuli in cells (Griswold & Adams, 1996; Wallace 1999). NSAIDs act by inhibiting cyclooxygenase (COX) enzymes that transform arachidonic acid (AA) from damaged membranes into eicosanoids, such as PGs, which are responsible for inflammation (Griswold & Adams, 1996). The right dorsal colon (RDC) is one of the sites where the harmful action of NSAIDs is demonstrated (Karcher, Dill, Anderson & King, 1990; Cohen, Carter, Mealey & Taylor, 1995; Galvin, Dillon & McGovern, 2004; Amaya & Flórez, 2011; El- Ashker, El-Khodery & Metwally, 2012; Andrade, Cassou, Aranzales & Alves, 2016). The mechanism for which confined ulceration and edema occurs in this portion of the large intestine is yet to be determined, but several hypotheses have been proposed in this regard (Tobin *et al*., 1986; Meschter, Gilbert, Krook, Maylin & Corradino, 1990; McCarthy, 1995; Barrison & Wolfe, 1999; Wallace, 1999; Cohen, 2002; Richter, R.-A., Freeman, Wallig, Whittem & Baker, 2002; McConnico, Morgan, Williams, Hubert & Moore, 2008). The diagnosis is essentially through clinical history of NSAIDs, clinical signs such as colic, anorexia, prostration, diarrhea, transabdominal ultrasonography (increased intestinal wall thickness, altered echogenicity), altered biochemical parameters such as hypoproteinemia and hypoalbuminemia by gastrointestinal loss, or through direct visualization of the RDC by celiotomy or necropsy (Cohen, 2002). The treatment can be medical and based on four pillars (avoid stress situations, discontinuation of NSAIDs, alteration of diet, and appropriate therapy, always keeping in mind the basic medical support) or surgical in chronic cases or failure in medical treatment (Hough, Steel, Bolton & Yovich, 1999; Galvin *et al*., 2004; Melo, Fiório, Araújo, Ferreira & Santos, 2009; Lane, Cohen, Zedler, Hollis & Southwood, 2010). The prognosis is reserved because usually the diagnosis is late (Jones, Davis & Rowlingson, 2003). However, there is an account of a horse that underwent a medical treatment of phenylbutazone for 15 days at the dose of 13,5 mg / kg IV, which survived medical treatment (Melo *et al*., 2009). There are, also, reports of horses that died with Right Dorsal Colitis (RDColitis) at relatively recommended doses and with short treatment periods (Cohen *et al*., 1995).

1. Anatomy of the gastrointestinal (GI) tract

As horses are non-ruminant, the stomach is also called as a simple stomach (Budras, Sack & Röck, 2011; Fails & Magee, 2018) and it has J shape (Sisson, 1975; Fails & Magee, 2018). This organ located caudal to the diaphragm and in the left side, is subdivided into cardia, fundus, body and pyloric region (Sisson, 1975; Fails & Magee, 2018). Both the cardia and the pyloric region have a functional muscle called sphincter, cardiac sphincter and pyloric sphincter, which prevent the food from retreating (the horse does not vomit) (Krunkosky, Jarrett & Moore, 2017) and controls gastric emptying for the most distal part of the gastrointestinal tract, respectively (Fails & Magee, 2018). The stomach, positioned on the left side with the greater curvature on the left costal face and the smaller curvature on the middle axis of the body, culminates in the first segment of the small intestine, the duodenum (Krunkosky *et al*., 2017; Fails & Magee, 2018).

The intestine of the horse consists in a thinner portion designed by Small Intestine and a larger portion designed by Large Intestine (Sisson, 1975; Budras *et al*., 2011). As mentioned before, the first is formed by the duodenum (Sisson, 1975; Budras *et al*., 2011; Krunkosky *et al*., 2017) which binds to the portion of the small intestine with greater length: jejunum (Fails & Magee, 2018). Most of jejunal coils are located in the left dorsal abdomen where they are mixed with descending colon (Sisson, 1975; Krunkosky *et al*., 2017). The ilium is the shorter part of the small intestine (Fails & Magee, 2018) and that through the ileocecal fold, ileal orifice, it flows into the cecum (Budras *et al*., 2011).

The horse has the largest and most complex large intestine of any of the domestic animals (Fails & Magee, 2018). The Large Intestine is composed of cecum, colon (ascending, transverse and descending parts) that terminates as the rectum and anal canal (Sisson, 1975; Budras *et al*., 2011; Krunkosky *et al*., 2017; Fails & Magee, 2018).

The cecum, with 1m or more in length and 30-35 liters (L) capacity, is highly expansive and is divided into base, body and apex (Sisson, 1975; Budras *et al*., 2011; Krunkosky *et al*., 2017; Fails & Magee, 2018). Of the four bands (lateral, medial, ventral and dorsal) constituting and forming the cecal sac, the lateral band originates the cecocolic valve, where it marks the beginning of the second part of the GI tract: the colon (Budras *et al*., 2011; Krunkosky *et al*., 2017).

The colon is constituted for ascending (large), transverse and descending parts (Budras *et al*., 2011). The large colon with 4m length and accommodating 80L of capacity or more, is composed by four parts: ventral right and ventral left, dorsal left and dorsal right, respectively continuous (Sisson, 1975; Budras *et al*., 2011; Krunkosky *et al*., 2017). The right ventral colon

leaves the cecum, runs cranially to the sternal flexure and turning through 180º for left and continuous, caudally, as left ventral colon (Freeman, 2002a; Budras *et al*., 2011; Krunkosky *et al*., 2017; Fails & Magee, 2018), forming a first horseshoe (Krunkosky *et al*., 2017; Fails & Magee, 2018). The left ventral colon continues caudally along the ventral abdominal floor and in the vicinity of the pelvis entrance turns 180º for above (the pelvic flexure) and becomes the left dorsal colon (Freeman, 2002a; Budras *et al*., 2011; Krunkosky *et al*., 2017; Fails & Magee, 2018), just dorsal to the left ventral colon (Fails & Magee, 2018). The left dorsal colon continues cranially, dorsally to the left ventral colon, follows as dorsal diaphragmatic or diaphragmatic flexure and succeeds as the RDC, on the right side (Freeman, 2002a; Budras *et al*., 2011; Krunkosky *et al*., 2017; Fails & Magee, 2018), forming a second horseshoe (Yildiz, Yildiz, Arslan & Õzgür, 2001; Krunkosky *et al*., 2017). Thus, there is a double horseshoe, one on top of the other, with the toes pointed cranially (Krunkosky *et al*., 2017). The different parts of the ascending colon differ in the number of taenia: all the ventral colon has 4 bands, the pelvic flexure is composed of one taenia as well as the left dorsal colon and the RDC has three (*taenia coli*) (Sisson, 1975; Yildiz *et al*., 2001; Nasr, Fadel, Noha & Elzanaty, 2014). The dorsal parts of the colon are deprived of the sacculations and constriction (Sisson, 1975), but Yildiz, *et al*., (2001), state that RDC it is composed of 3 sacculations (*haustra coli*).

After the RDC, follow the transverse colon, which as its name indicates crosses the body from the right side to the median plane and becomes the descending/small colon (Sisson, 1975; Budras *et al*., 2011; ; Krunkosky *et al*., 2017; Fails & Magee, 2018), also occupying the left flank with the jejunum (Budras *et al*., 2011), dorsally the left parts of ascending colon (Sisson, 1975).

Upon arriving at the pelvic entrance, the descending colon gives place to the last part of the intestinal tract, the rectum that through the anus communicates with the exterior (Sisson, 1975; Budras *et al*., 2011; Krunkosky *et al*., 2017).

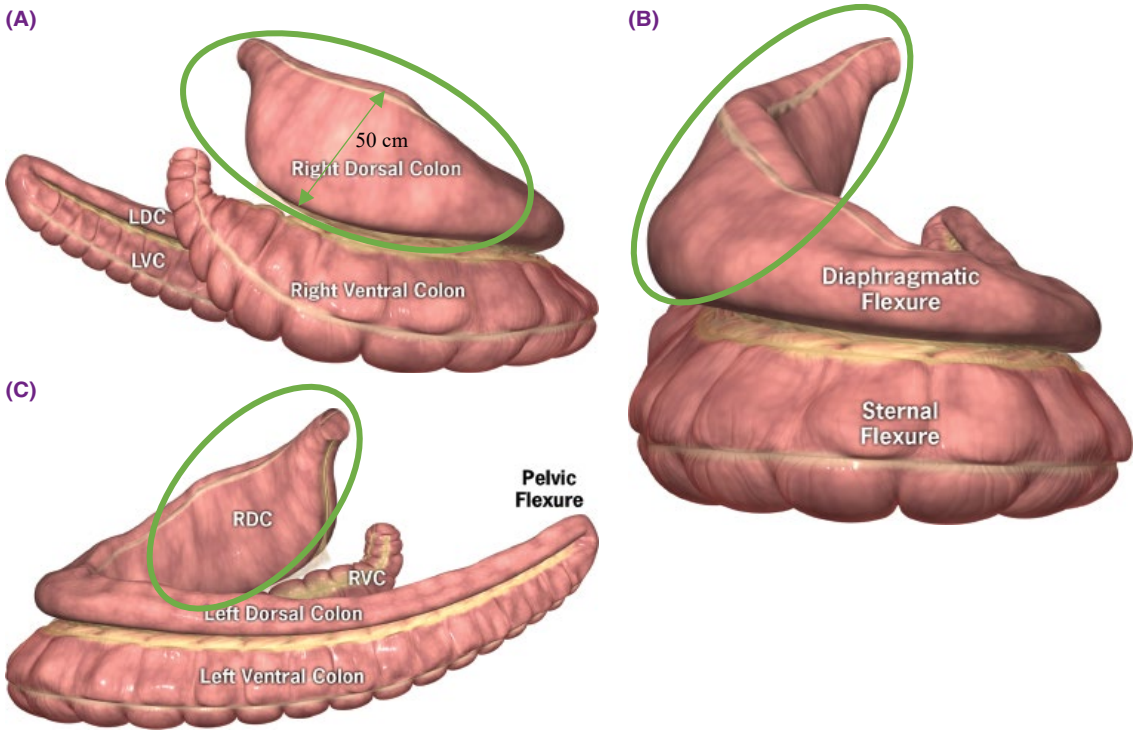
2. Right Dorsal Colon

2.1 Anatomy review

The RDC originates from the diaphragmatic flexure and extends caudally to the base of the cecum on the right side and binds to the transverse colon (Budras *et al*., 2011), dorsal to the right ventral colon (Sisson, 1975; Krunkosky *et al*., 2017). Throughout the colon, the diameter varies. The RDC has the greater diameter of all ascending colon (ampulla coli), reaching 50cm of diameter, whit three longitudinal bands (*taenia coli*) and lack sacculations (smooth) (Sisson, 1975; Budras *et al*., 2011; Krunkosky *et al*., 2017), namely three wrote Yildiz, *et al*., (2001).

Knowledge of the anatomical location of the RDC within the abdominal cavity is important for evaluation by the outside. In a study by Amaral and Froes, (2014), the RDC was found by ultrasonography in 100% in the upper right flank areas from caudal to the last rib to the 15th intercostal space (ICS) and in the middle third of the rib of the 10th to 12th ICS.

Figure 6- Large colon of the equine Adapted: Courtesy of The Glass Horse, Science In 3D)



(A)- The large colon, as viewed from right side of the horse. The left ventral (LVC) and left dorsal (LDC) colons are evident towards the caudal aspect of the horse's abdomen. RDC (green) has an average of 50cm of diameter. (B)- the large colon from cranial-most aspect of the abdomen, depicting the sternal flexure in the ventral colon and the diaphragmatic flexure in the dorsal colon. (C) The large colon, as viewed from the left side of the horse. The right ventral (RVC) and right dorsal (RDC- green) colons are identified.

2.2 Intestinal ultrastructure

Colon has a basics structural features that they are common all regions of the GI tract. The GI tract is a tube composts for hallow lumen with a wall made up of four layers (from the inside to the outside): mucosa, submucosa, muscularis and serosa (Sisson, 1975; Freeman, 2002a; Freeman, 2003; Frappier, 2006; Hendrickson, Malone & Sage, 2007; Junqueira & Carneiro, 2013; Mescher, 2016; Fails & Magee, 2018). The mucosa (that communities with lumen) of the colon is composed of three histological layers: (a) simple columnar epithelium, (b) a lamina propria of loose connective tissue with blood and lymphatic vessels and smooth muscle cells and a (c) muscularis mucosae, the outer border of the mucosal layer with two sub-layers of

smooth muscle cells, the innermost circular and the outermost longitudinal (Junqueira & Carneiro, 2013; Mescher, 2016; Fails & Magee, 2018). Due to the local movement promoted by the two mentioned sub-layers, the contact of the mucosa with the ingesta increase (Junqueira & Carneiro, 2013; Mescher, 2016).

Next layer, submucosa, is a denser connective tissue with larger blood and lymphatic vessels and contains Meissner (submucosal) plexus of automatic nerves (Junqueira & Carneiro, 2013; Mescher, 2016; Fails & Magee, 2018).

The muscularis is responsible for the motility of the intestine, and is therefore constituted by smooth (involuntary) muscle organized also, in two layers, one internal circular and one external longitudinal (Sisson, 1975; Junqueira & Carneiro, 2013; Mescher, 2016; Fails & Magee, 2018) and, between the muscle sublayers has a Myenteric (Auerbach) nerve plexus (Junqueira & Carneiro, 2013; Mescher, 2016).

The serosa also called visceral peritoneum is continuous by mesenteries that supports the intestine (Junqueira & Carneiro, 2013; Mescher, 2016) and it is constituted by loose connective tissue, blood and lymphatic vessels and adipose tissue (Junqueira & Carneiro, 2013; Fails & Magee, 2018).

The four layers can be distinguished by ultrasound because they have different echogenicity (Freeman, 2003; Jones *et al.,* 2003; Hendrickson *et al*., 2007), but five layers are viewed ultrasonographically, the additional one being designated by mucosal interface (Reef, 1998; Freeman, 2002a; Freeman, 2003). This one results from gas and ingesta on the surface of the mucosa and is present in both small and large intestine (Freeman, 2002a).

The mean normal thickness of the RDC was 3,4 ± 0,55 mm in the control time of the study Andrade, *et al.*, (2016), varying from 3,4mm in 10th ICS to 4,2mm in 14th ICS in the healthy horses in study by Jones *et al*., (2003), approximately 3mm in thickness (Reef, Whittier & Allam, 2004), 1,6mm to 2,7mm in the study by Hendrickson *et al*., (2007), 3mm in the book by Reef, (1988), 4mm to 9mm not distended and 2mm to 4mm when distended or averaged 3mm when distended and 5mm when nondistended in the study by Fleischer, Muhletaler and James, (1981), in a study of Pinto *et al*., (2010), the mean was 1,6 ± 0,7mm, 2,6 ± 0,4 mm in the study of Amaral and Froes, (2014), and 3,7 ± 0,3mm (Thoroughbred) in the study by Siwinska, Zak, Baron, Cylna and Borowicz, (2017). Reef, (1998), states that the intestinal wall should not exceed 4mm thick, so as not to be considered pathological. Physiology, the size of the animal (Freeman, 2002a; Siwinska *et al*., 2017), the degree of distention (Fleischer *et al*., 1981; Freeman, 2002a), can cause changes in the thickness of the wall of the intestine, and, physically, also the pressure exercised by the probe (Freeman, 2002a).

Figure 7- Layers of a typical segment of gut (Adapted: Fails & Magee, 2018)

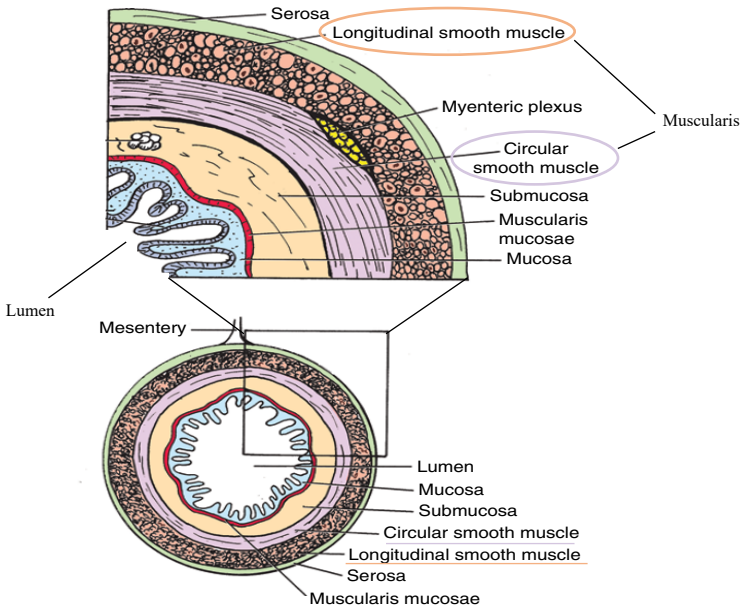
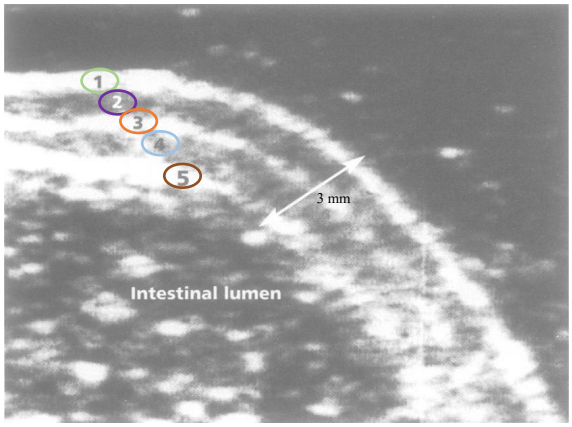


Figure 8- Magnified ultrasonogram of small intestine imaged in a water bath using a 10MHz linear transducer, showing the five layers of the intestinal wall (Adapted: Freeman, 2002a)



Legend: 1- Serosa; 2- Muscularis; 3- Submucosa; 4- Mucosa; 5- Mucosal Interface. The total Wall thikness (double arrow) was 3mm.

2.3 Physiology

The first two parts of the Large Intestine (cecum and colon) allow stasis of ingestion for microbial destruction because they have expansive properties (Budras *et al.*, 2011). Here there are the functions of mix, secretion and absorption of VFAs (Volatile Fatty Acids) and fluids through the colonic mucosa to blood (Argenzio, Southworth & Stevens, 1974b; Gurtler, Ketz, Kolb, Schröder & Seilel 1976). In the large intestine the cellulose is degraded due to the action of the fermenting bacteria existing in this compartment and produce volatile fatty acids, as

acetic acid, propionic acid and butyric acid (Gurtler *et al*, 1976; Argenzio, 1981; Argenzio & Meuten, 1991). The final products of fermentation are rapidly absorbed by colonic mucosa (Gurtler *et al*, 1976: Argenzio & Meuten, 1991) in the order of acetate, propionate and butyrate (Argenzio *et al*., 1974b).

Both the cecum and the ascending colon are sites of fermentation and microbial digestion of cellulose that may take days (Fails & Magee, 2018). Movements of these organs mix the contents to allow fermentative digestion and to expose the contents to the epithelial surface for absorption of volatile fatty acids (Fails & Magee, 2018).

3. Methods of evaluation of the RDC

Ultrasonography is a diagnostic tool used by the medico-veterinary practice to evaluate intraabdominal organs, including locating the RDC, visualizing the different layers and measuring the total thickness of the intestine wall (Freeman, 2002a; Amaral & Froes, 2014).

The knowledge of the abdominal anatomy and ultrasound characteristics is essential for the interpretation of changes that come with the diseases (Freeman, 2002a). Indeed, changes in the diameter, contents, location, motility and thickness of the intestine wall should be noted (Freeman, 2003). Thus, ultrasonographic evaluation of the GI tract involves assessment of intestinal wall thickness, organ distension, and motility, and luminal contents (Reef, 1998; Freeman, 2002a; Freeman, 2002b; Reef *et al*., 2004).

Ultrasonography nowadays has better resolution (Freeman, 2002b), and it can detect anatomical (displacement, twisting) and inflammatory (enteritis, colitis) abnormalities (Reef, 1998; Andrade *et al*., 2016). Several studies have already demonstrated the reliability of transabdominal ultrasound to assess abdominal structures (Jones *et al*., 2003; Amaral & Froes, 2014), including assess the thickness and regularity of the bowel wall (Fleischer *et al*., 1981).

The transcutaneous technique dictates that the animal's coat has to be clipping, the skin clean and the coupling gel applied (Freeman, 2002a; Reef *et al*., 2004). When there is no possibility of clipping the hair, one must wet the hair with alcohol in the direction that it normally lies (Freeman, 2002a; Reef *et al*., 2004), although the quality of the image is greater when there is hair clipping and subsequent cleaning of the skin (Williams, Cooper & Freeman, 2014). The transducer depends on the size of the animal, but usually, frequencies between 2.5-5 MHz, convex or sectional are appropriate (Freeman, 2002a). In a study of Amaral and Froes, (2014), frequencies 2,5-6,6 MHz was used with convex probe, and a study by Hendrickson *et al*., (2007), abdominal ultrasonography examination was performed by using 5MHz sector transducer. High frequencies (5-10 MHz) have a higher resolution but lower depth of beam

penetration (with a high frequency have minimal ability to penetrate deep into biologic tissue), indicated for surface structures or for horses with less body fat and for foals (Freeman, 2002a; Smith & Fry, 2004). Low frequencies (2.5-3.5 MHz) will penetrate with greater depth (up to 30cm) and reach deeper internal organs or obese patients, however, with a lower image quality than the high frequency produced (Freeman, 2002a; Smith & Fry, 2004).

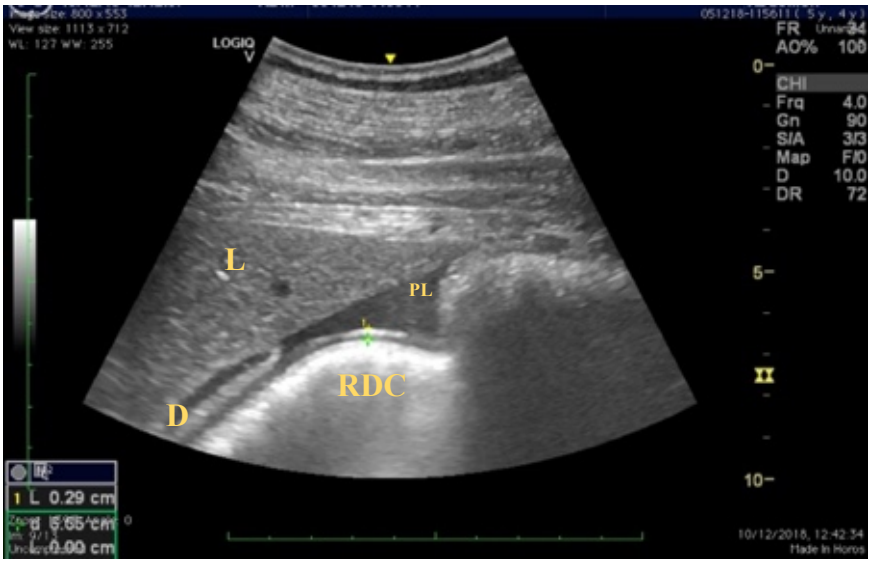
The angle of the probe must be 90º with the intestinal wall to visualize the structures as well as possible (Freeman, 2002a).

Based on anatomical knowledge, the RDC can be found through ultrasound on the right flank. The RDC has a smooth nonsacculated appearance (Reef *et al*., 2004). The RDC can be 100% identified in the regions of the right upper flank from caudal to the last rib to the 15th ICS and in the middle third of the costal arch between the 10th to 12th ICS (Amaral & Froes, 2014). In a study by Jones *et al*, (2003), the RDC was consistently visualized in the 11th, 12th, and 13th ICS and was sometimes possible the visualization in the 10th and 14th ICS.

There are structures that delimit the RDC: liver, lung and duodenum. The liver, on ultrasound, is dorsal/axial to the RDC, and the margin of the lung is above the RDC (Jones *et al*, 2003). The duodenum is found in the same sonographic areas of the RDC, between the right portion of the liver and the RDC (Amaral & Froes, 2014), from 12th to 15th ICS (Freeman, 2002a; Scharner, Rotting, Gerlach, Rasch & Freeman, 2002; Nasr *et al*., 2014).

The presence of sacculations is characteristic of the ventral colon and is therefore an auxiliary method of distinction between the ventral and dorsal portion of the ascending colon (Freeman, 2002a; Amaral & Froes, 2014; Nasr *et al*., 2014). Hendrickson *et al*., (2007) proposed that the possible sacculations that RDC presented ultrasonographically, could be due to the peristaltic movements of the intestine. The fact that RDC appears with or without sacculations, is still unclear, but in comparison to the ventral colon, this has much more evident sacculations (Williams *et al*., 2014).

Figure 9- Transabdominal ultrasound of the RDC, with the duodenum and liver as reference points.



Legend: L, Liver; D, Duodenum; PL, Peritoneal liquid; RDC, Right Dorsal Colon.

3.1. Advantages and limitations on the evaluation of the RDC by ultrasonography

Ultrasonography abdominal is characterized by being a non-invasive imaging method with minimal risk of complications, is readily performed when appropriate equipment is available, is easy to perform and provides real-time (dynamic) information about the structure examined (Reef, 1998; Nasr *et al.*, 2014; Siwinska *et al.*, 2017;) as well as accompanying the evolution of the clinical situation of the animal (Reef *et al.*, 2004; Davis, 2017; Biscoe, Whitcomb, Vaughan, Dechant & Magdesian, 2018). It can be useful in the decision for surgical or medical treatment (Freeman, 2002b; Scharner *et al.*, 2002; Reef *et al.*, 2004). Additionally, this is well tolerated by animals, and sedation is not mandatory (Reef *et al.*, 2004).

The interpretation of the image is very dependent on the experience and skill of the operator (Jones *et al.*, 2003; Galvin *et al.*, 2004). The gas within the lungs and the gas and ingesta within the large intestine causes reverberation artifact and acoustic shadow that reflect most or all of the ultrasound beam, making it difficult to visualize the sub-structures and some lesion which are beneath these organs and limit assessment of the intestinal lumen and far wall (Freeman, 2002a; Amaral & Froes, 2014; Williams *et al.*, 2014; Biscoe *et al.*, 2018).

To accommodate large-capacity organs (Budras *et al*, 2011), the abdominal cavity of the horse becomes large and deep to be accurately examined (Scharner *et al.*, 2002; Freeman, 2003; Siwinska *et al.*, 2017). The size and depth of the equine abdomen is a limitations (Scharner *et al.*, 2002; Freeman, 2003; Williams *et al.*, 2014; Siwinska *et al.*, 2017), however, the new

abdominal ultrasound windows studied by Amaral and Froes, (2014), “minimized” the size of the abdominal cavity as a limitation, and simultaneously, provided a greater and better visualization of the RDC.

The inherent mobility of some structures within the abdominal cavity may hamper ultrasound examination (Williams *et al*., 2014).

As a diagnostic test for right dorsal colitis (RDColitis), the portion of the affected colon may be not visualized (Jones *et al*., 2003). Due to its 50cm diameter and large lumen, only the parietal side can be examined by ultrasound, resulting in false negatives in cases of focal RDColitis of the inaccessible visceral face (Cohen, 2002; Galvin *et al*., 2004; Reef *et al*., 2004; Andrade *et al*., 2016). A horse of the study by Galvin *et al*., (2004), have no evidence of thickening of the RDC by abdominal ultrasound and two weeks later underwent a celiotomy and visual inspection confirmed the RDColitis. However, RDColitis tends to be diffuse and in the study by Andrade *et al*., (2016), it was not a problem in its detection.

A horse may have ulceration of RDC and have a thickness within the normal range, however it does not have this healthy colon segment (Jones *et al*., 2003).

Obesity worsens the quality of the ultrasound image (Scharner *et al*., 2002; Amaral & Froes, 2014; Biscoe *et al*., 2018) because the fat absorbs the sound waves, decreasing their propagation and causing loss of image quality (Amaral & Froes, 2014; Williams *et al*., 2014). Smith & Fry, (2004) wrote as ultrasound travels through tissue, the strength of the ultrasound energy is attenuated. In a study by Pinto *et al*., (2010), showed that image quality was affected by obese or overweight horses.

Besides that, in a healthy RDC is difficult to differentiate between the various layers, since the hyperechogenic mucosa is indistinguishable from the contents of the colon (Jones *et al*., 2003).

3.2. Basic Ultrasound Concepts

Structures that reflect all the ultrasonic beams appear to the monitor as bright white lines (hyperechoic) (Smith & Fry, 2004). Structures that poorly reflect the ultrasound beams appear in darker shades of gray (hypoechoic) (Smith & Fry, 2004). Structures that do not reflect any ultrasound beam appear such as black to the monitor (anechoic) (Smith & Fry, 2004).

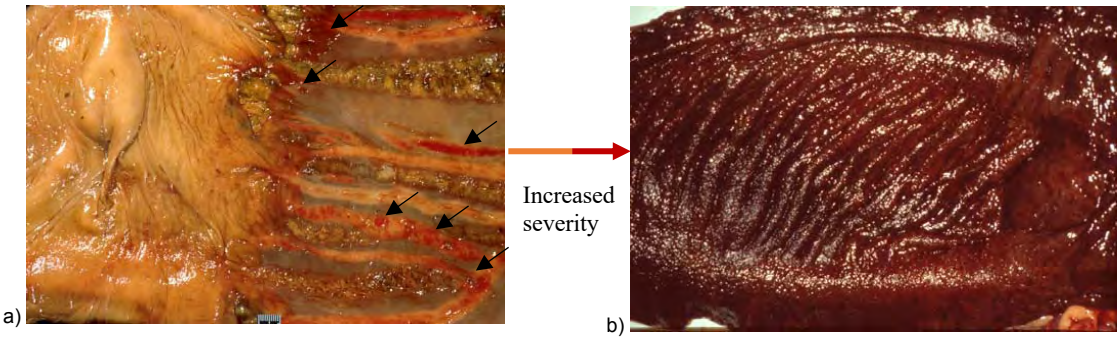
The large intestinal wall is hypoechoic to echogenic and the mucosal surface is hyperechogenic (Reef, 1998; Reef et al., 2004). The colon wall thickness measures 3-4mm or less and appears homogeneously hyperechoic (Jones *et al*., 2003). The ultrasonographic layers are hyperechoic serosa, hypoechoic muscularis, hyperechoic submucosa, hypoechoic mucosa and hyperechoic mucosal interface (Reef, 1998; Freeman, 2002a).

4. Right Dorsal Colon disorders

4.1 Right Dorsal Colitis

RDColitis, in general, is a specific and confined inflammatory enteropathy of the RDC of the equine, ulcerative, edematous, and protein-losing type described a long time ago to this day, associated with history of non-steroidal anti-inflammatory drugs (NSAIDs), especially phenylbutazone (PBZ) (Karcher *et al*., 1990; Cohen *et al*, 1995; Galvin *et al*, 2004; Jones *et al*., 2003; Amaya & Flórez, 2011; El- Ashker, El-Khodery & Metwally, 2012). The disease has been observed following high dose phenylbutazone administration (Karcher *et al*., 1990; Andrade *et al*, 2016; McKenzie III, 2017), but the toxic effects of PBZ do not always correlate closely with doses of the drugs (Cohen *et al*., 1995; Galvin *et al*, 2004; McKenzie III, 2017). In the study by Cohen *et al*., (1995), RDColitis has been documented on horses receiving normal dosage of PBZ. The pathophysiology is not yet clear (Karcher *et al*., 1990; Cohen, 2002; Rithcer, Freeman, Wallig, Whittem & Baker, 2002; Davis, 2017), however there are several plausible hypotheses that are being studied.

Figure 20- Ulcerative lesions of the right dorsal colon secondary to NSAID toxicosis in two horses (Adapted: Davis, 2005)



a) Illustrates the transition from normal epithelium to the severely ulcerated epithelium. b) Diffuse ulceration of a severely affected colon.

4.1.1. Pathophysiology

It is believed that there are systemic and local factors that contribute to the development of RDColitis (Tobin *et al*., 1986; Meschter, *et al*., 1990; McCarthy, 1995; Barrison & Wolfe, 1999; Cohen, 2002; McConnico *et al*., 2008), however, the exact reason for colitis only in this portion of the large intestine remains to be determined (Davis, 2017).

The mechanism of action whereby NSAIDs lead to intestinal damage is via cyclooxygenase (COX) blockade and subsequent inhibition of eicosanoid formation, from the AA pathway

(Griswold & Adams, 1996; Marshall & Blikslager, 2011). PGE, PGF and PGI, are responsible for maintenance of normal mucosal function and health (Davis, 2017, McKenzie III, 2017). In the colon, PGE_2 was the predominant PG (Meschter *et al*., 1990). Several nonsteroidal anti-inflammatory drugs (NSAID) have been demonstrated to possess ulcerogenic properties in the alimentary tract of horses (Karcher *et al*., 1990; MacAllister, Morgan, Borne & Pollet, 1993; Noble *et al*., 2012), may result from inhibition of PGE_2, which seems to act locally to increase mucosal blood flow (Lees & Higgins, 1985; Geor, Petrie, Papich & Rousseaux, 1989). Blikslager, Roberts, Rhoads & Argenzio, (1997), wrote in your study that primary function of these compounds is maintenance of normal mucosal blood flow and tight-juntion functionality. NSAIDs disrupts these functions and results in mucosal barrier dysfuntion and injury, with consequent secretory diarrhea, hypoproteinemia and hypoalbuminemia, in association with mucosal ulceration, neutrophilic inflammation and colon wall edema (Karcher *et al*., 1990; Cohen *et al*., 1995; Andrade *et al*, 2016). It was considered that there was a decrease in blood flow to the GI mucosa, since PG production was inhibited and inability to restore the mucosal barrier (Marshall & Blikslager, 2011). Meschter *et al*., (1990) and McConnico *et al*., (2008) however, here is no evidence of significantly reduced PGE_2 production in mucosal biopsies in the RDC. McConnico *et al*., (2008) have shown that long phenylbutazone therapies increase arterial blood flow in this segment of large intestine, but the real reason for this outcome is also unknown. It can probably indicative of a colonic generalized acute inflammatory response with vasodilation and the possibility of angiogenesis (McConnico *et al*., 2008; Davis, 2017).

Oxidative stress may play a role in ulcer development. Aranzales, Andrade & Alves, (2014), showed in a study in which PBZ induced oxidative stress on glandular gastric mucosa in horses, altering the antioxidant-oxidant balance of their surface. This may be another mechanism of mucosal injury by NSAIDs.

Decreased nitric oxide caused by phenylbutazone was also found in the study by Barrison & Wolfe, 1999, which together with PGE_2 play a protective role in GI mucosa.

Another hypothesis is that the RDC is the only section of the colon with net water secretion, and this unique activity may predispose this section of colon to disease (Argenzio, Lowe, Pickard & Stevens, 1974a; El- Ashker *et al*., 2012). In this study it was proposed that there may be pathways of oxidative stress injury that originate colitis and gastritis. When there is oxidative injury of the RDC in horses, a migration of eosinophils, that were located near the muscularis mucosae, to the surface of the mucosa (lumen surface), which may be an important factor in the pathophysiology of the disease (Rötting, Freeman, Eurell, Constable & Wallig, 2003).

It was proposed in the study by Maitho, Lees & Taylor, (1986), that oral phenylbutazone could be dissolved primarily in the gastrointestinal fluids. The release of phenylbutazone, slow release

from binding sites in the RDC, from the adhered roughage can only occur in the fermentative deposits (colon and cecum) where the fibrous constituents of the cellulose are broken, and then it is absorbed by the mucosa of the large intestine (Lees & Higgins, 1985; Mathio *et al*., 1986; Tobin *et al*., 1986). Junction between the dorsal colon and small colon appeared to serve one of the major barriers to digesta flow, resulting in resistance to digesta flow (Argenzio *et al*., 1974a). This narrowing of the lumen at the junction of the RDC and the transverse colon, and prolonged intestinal transit time increases the NSAID contact time with the mucosa of the colon providing an environment conducive to the development of inflammation and ulceration - topical irritation (Cohen, 2002).

With the loss of colonic mucosal integrity (increased colonic permeability) caused by NSAIDs, a more marked plasma protein loss occurs, bacterial flora may invade the damaged mucosa, and bacterial endotoxin (such as LPS) may be absorved leading to further compromise of the patient (East *et al*., 2000; Campbell, Jones & Blikslager, 2002). Richter *et al*, (2002), demonstrated in vitro that PBZ interferes in mucosal integrity and anion transport systems in the RDC through the accumulation of this substance around the epithelial cells, as would be expected in vivo by diffusion from capillaries. Bjarnason, Fehilly, Smethurst, Menzies & Levi, (1991), have shown that certain NSAIDs may increase the permeability of the intestinal wall to the small intestine of humans. They describe that the systemic effects mediated by NSAIDs are less important, "are reactively weak," and enhance local effects after absorption or after biliary excretion of the drug.

4.1.2. Predisposing factors

The horses most commonly affected with this condition are younger horses, because they are more likely to be in performance horse and more likely to require NSAID's administration for lameness for example or other musculoskeletal disorders (Lees & Higgins, 1985; Karcher *et al*., 1990; Cohen *et al*., 1995; Cohen, 2002; Galvin *et al*., 2004). Additionally, they are subject to stress and dehydration due to transport and exercise (Cohen, 2002). Another predisposing group are ponies and miniature horses where their weight is overestimated which results in inappropriately high doses (Karcher *et al*. 1990; Cohen, 2002; Davis, 2017). Although, toxicity is related to dose and duration of administration, some horses develop intoxication at the recommended therapeutic doses (Cohen *et al*., 1995; Melo *et al*., 2009). NSAID administration to hypovolemic horses or to horses concurrently deprived of water may potentiate the toxic manifestations of these pharmaceuticals by eliminating the prostaglandin-mediated local vascular changes that guard against ischemic injury (Gunson & Soma, 1983; Karcher *et al*.,

1990). In a study by Hough *et al*., (1999), one horse after exercise was dehydrated and received NSAIDs (twice the recommended dose) that may have boosted RDColitis (phenylbutazone toxicosis may have been aggravated by dehydration). In 1990, a study of Karcher *et al*., have been produced ulceration of RDC, in as a little as 5 days, whit high doses of phenylbutazone oral (6g PO once day for 5 day) with concurrent 50% restricting water intake. This suggests that the administration of NSAIDs to dehydrated animals is a risk factor (Jones *et al*., 2003). Other predisposing factor are infection, immune-mediated response, behavioral traits, genetic factors and stress (Karcher *et al*., 1990) and enterotoxaemia or preexisting lesions of the colon, or renal or hepatic disease and septicemia, increase the risk of RDColitis in horses treated with NSAIDs, because they cause reduction of tissue perfusion and elimination of the drug (Galvin *et al.*, 2004; Melo *et al*., 2009). The variable occurrence of the toxic side effects of phenylbutazone at daily doses recommended may be attributed to individual variation (Jones *et al*., 2003)., i.e., age, breed, health status, stress levels, hydration or diet (type of protein), duration of treatment, as well high doses and administration of concomitant drugs (Lees & Higgins, 1985; Karcher *et al*., 1990; Meschter *et al*., 1990; Cohen *et al*., 1995; Richter *et al*., 2002; Galvin *et al.*, 2004). Melo *et al*., (2009), writes that doses up to 8,8 mg / kg can be used without risks during short periods of treatment, but there are differences in susceptibility to intoxication that vary according to breed, duration of treatment, administration and formulation of the drug.

4.1.3. Causes

In the study by Lees and Higgins, (1987), it is reported that in 1979, Snow and others showed that NSAIDs could have toxic potential and even death for ponies. Since then, studies have been developed with the purpose of discovering the reason why the sensitivity of the dorsal colon right to this type of drug. History of administration of NSAIDs prior to the development of clinical GI abnormalities is suggestive of a causal relationship between administration of these drugs and subsequent colitis (Karcher *et al*., 1990; Hough *et al*., 1999). PBZ is the drug most commonly reported in the induction of RDColitis, but Flunixin meglumine, Ketoprofen, and Meloxicam are also described (Karcher *et al*., 1990; MacAllister *et al*., 1993; Cohen *et al*., 1995; Noble *et al*., 2012). Multiple reports of experimental NSAID toxicity are available in the literature (Meschter *et al*., 1990; MacAllister *et al*., 1993; McConnico *et al*., 2008; Mozaffari, Derakhshanfar, Alinejad & Morovati, 2010) and clinical presentation in these reports varied greatly from none detectable to sudden death due to colon rupture and septic shock (severe

ulceration of the colon provides localized areas where endotoxins and luminal pathogens can easily enter the vascular system, leading to systemic infection and septicemia).

4.1.3.1. AINE's

Inflammation results from a cell lesion of variable origin (Griswold & Adams, 1996; Tasaka, 2014; Cole, 2015). Dispersed by the healthy organism, the receivers cells of the lesion stimulus (resident macrophages, detrital cells and mast cells) release various inflammatory mediators, such inflammatory cytokines (IL-1α, IL-1β and TNFα) (Brzozowski *et al*., 2001; Tasaka, 2014) responsible for the 5 signs of inflammation: redness and heat (by vasodilation, which results in increased blood flow), swelling (by increased permeability of the blood vessels, which results in exudation of plasma proteins and fluid), pain and function laesa (loss of function) (Griswold & Adams, 1996; Tasaka, 2014; Cole, 2015). Cell membranes are composed of organized phospholipids. At the time of injury, the affected cells release the membrane phospholipids that are converted to AA by phospholipase A2 and hydrolases (Radi & Khan, 2006; Tasaka, 2014; Cole, 2015). Arachidonic acid is the substrate for the formation of eicosanoids, which are unsaturated lipids (biologically active lipids) that through the action of specific enzymes such as cyclooxygenase (COX) and lipoxygenase (LO) that are fundamental for the inflammatory process (Griswold & Adams, 1996; Tasaka, 2014; Cole, 2015). The COXs, integral membrane proteins, originate prostanoides, including prostaglandin (PGE_2, PGD_2, $F_{2\alpha}$, I_2) and thromboxane A_2 and LO originate leukotrienes (LT) (Griswold & Adams, 1996; Tasaka, 2014; Davis, 2018). PGs cause, mainly arteriolar vasodilation, induces increased vascular permeability and edema (Tobin *et al*., 1986; Griswold & Adams, 1996; Wallace 1999; Tasaka, 2014). Prostaglandins have been shown to restore barrier function of ischaemic-injured intestinal mucosa, possibly via an action on interepithelial tight junctions (Blikslager *et al*., 1997). Thromboxanes are vasoconstrictors and a potent platelet aggregation and therefore antagonistic to PGs (Radi & Khan, 2006; Tasaka, 2014).

Used as therapeutic resources for pain relief and inflammation in horses, this pharmacological group inhibits the enzyme COX, through inhibition of arachidonate binding and suppress prostaglandin synthesis (Lees & Higgins, 1985; Tobin *et al*., 1986; Griswold & Adams, 1996; Wallace 1999; Cohen, 2002; Davis, 2006; Soma *et al*., 2011; David, 2018).

PBZ was introduced into veterinary medical practice in the 1950s (Tobin *et al*., 1986; Santos *et al*., 2009) and still remains one of the more commonly used NSAIDs in the horse (Lees & Higgins, 1985; Tobin *et al*., 1986; Lees & Higgins, 1987; Tomlinson & Blikslager, 2003; Reed, Messer IV, Tessman & Keegan, 2006; Davis, 2017; McKenzie III, 2017). Phenylbutazone

(PBZ) is the agent that is most prescribed for the relief of musculoskeletal pain (acute or chronic, mild to moderate) and inflammation, while Flunixin Meglumine is the most correct option for colic pain, fever, soft tissue inflammation, less frequent, for musculoskeletal problems as well has the anti-endotoxin properties (neutralizes the systemic effects of endotoxin) (Lees & Higgins, 1985; Santos *et al*., 2009; Cole, 2015) with a greater safety margin (Lees & Higgins, 1985).

There are three different types of COX, however the COX-3 function on the horse is still unknown (Tasaka, 2014; Davis, 2018). COX-1, present in most normal tissues, such as platelets, kidneys and gastrointestinal tract is considered constitutively expressed and responsible for PG production during normal physiological processes (Cole, 2015; Davis, 2018) and COX-2, are expressed at only low levels in normal tissue (Cole, 2015; Davis, 2018). The COX-1 is important in maintaining mucosal epithelial heath through adequate blood flow, epithelial cell turnover, bicarbonate production and mucous secretion", thus, it is only possible with basal physiological levels of COX (Davis, 2006).

In the review article by Davis, (2017), more consistent adverse events induced by NSAIDs were colic, diarrhea, weight loss, depression, lethargy and anorexia, edema, fever and death, and the parenteral route was responsible for most of the side effects. It is believed to be reasonably tolerate in horses when administered at the recommended dosage and dosing interval, but adequate therapies have been shown to cause adverse effects such as gastric ulcers, renal dysfunction (pappilary necrosis) and ulceration and inflammation of the mucosa of the RDC (Karcher *et al*., 1990; MacAllister *et al*., 1993; Cohen *et al*., 1995; Mozaffari *et al*., 2010).

Phenylbutazone was considered the most potently toxic NSAID, followed by flunixin meglumine and then ketoprofen (MacAllister *et al*., 1993; Mozaffari *et al*., 2010). The absence of significant protein loss in the Mozaffari *et al*., (2010), study may have been due to the drug given twice a day instead of four times. In the Davis review paper, 2017, ketoprofen was associated with milder adverse signs of NSAID toxicity. Concomitant administration of two type of NSAIDs, was been to be devastating for an equine in the study by Reed *et al*., 2006. Karcher *et al*., (1990), already stated that the therapeutic combination with at least two NSAIDs predisposes right dorsal colitis.

Both NSAID overdoses and duration of therapy over time are factors that increase NSAID toxicity (Hough *et al*., 1999). It is important that the dose and the time of treatment be critically respected to avoid the toxicity documented by NSAIDs. PBZ has a recommended dose of 4,4 mg / kg IV, every 12 hours on the first day and then reduced by half (2,2 mg / kg IV) for a maximum period of 4 days (Amaya & Flórez, 2011). Coadministration of more than one NSAID is not recommended (Reed *et al*., 2006). Briefly, NSAIDs competitively inhibit the two

COX isoforms, that is, they inhibit the production of eicosanoids (Tasaka, 2014; Cole, 2015) Selective NSAIDs for COX2 (the main enzyme in the inflammatory process and minor in the homeostatic processes) are considered safer (Cole, 2015).

4.1.4. Clinical Signs

Signs of RDColitis are non-specific and variable, horses have, always, more than one clinical sign and can occur within days to weeks (Cohen, 2002). Differential diagnosis for clinical sings including: ulceration of the stomach and other portions of the intestine, sand enteropathy, cholelithiasis, cholangiohepatitis and other hepatopathies, inflammatory bowel disease (lymphocytic/plasmocytic enteritis) alimentary lymphosarcoma, larval cyathostomiasis, enterolithiasis and muscular hypertrophy of the ilium (Cohen, 2002).

In review article, Davis, (2017), wrote that colic and diarrhea are the most common clinical signs associated with disease, but there are other such depression, lethargy, anorexia, fever, edema and weight loss. Death or euthanasia in more 50% of the literature review cases (Davis 2017).

Colic can range from mild and intermittent due to chronic disease and colonic stricture (Hough *et al*., 1999) to severe colic with acute disease. The duration of clinical signs in horses with strictures is greater, may indicate that the condition progresses to stricture of the RDC (Cohen *et al*., 1995).

Acute clinical signs include apathy, inappetence, depression, prostration, lethargy, anorexia, fever, acute abdominal pain mild to severe, diarrhea, fever and eventually death (Tobin *et al*., 1986; Karcher *et al*., 1990, Cohen *et al*., 1995: Andrade *et al.*, 2016), reduced appetite, dehydration or hypovolemic shock and endotoxemia may occur along with all of its associated clinical signs (Karcher *et al*., 1990; Galvin *et al*., 2004; Davis, 2006). Jugular thrombophlebitis may also occur, evidencing a history of recent NSAID administration, because they are very irritating when injected extravascular (Tobin *et al*., 1986; Hough *et al*., 1999; Melo *et al*., 2009; Andrade *et al.*, 2016). Most NSAIDs administered via IV are irritating to muscle tissues (Cole, 2015), with perivascular reactions occurring (Lees & Higgins, 1985).

Chronic clinical signs include reduced feed intake, mild to moderate intermittent abdominal pain, soft to loose feces, weight loose and both ventral or peripheral edema resulting from protein-losing enteropathy, the duration of which can vary from weeks to several months (Karcher *et al*., 1990; Cohen *et al*., 1995; Hough *et al*., 1999; Cohen, 2002; Galvin *et al*., 2004; Davis, 2006; Reed *et al*., 2006; Melo *et al*., 2009; El- Ashker *et al*., 2012).

4.1.5. Gross Lesions

Horses with a treatment of phenylbutazone twice the therapeutic dose (13,46 mg / kg) did not observe macroscopic changes of the colon (Meschter *et al*., 1990). At 48 h after the initiation of treatment (2 treatments with phenylbutazone overdose) the entire RDC was markedly thickened and dark purple, 72 h (with three treatments of phenylbutazone sodium), the mucosa was severely congested, dark purple, edematous and numinously demarcated and at 96 h (with four treatment of phenylbutazone overdose) the colon mucosa contained extensive red-dark miliary foci (Meschter *et al*., 1990). Hemorrhage on the serosal surface, along with ulceration in the mucosa and oedema of the colon wall (Karcher *et al*., 1990). Oedema or thinking restricted in the RDC and diffuse or multifocal ulceration of the mucosal surface (Cohen, 2002). Multifocal areas of congested serosa, old and fresh, with irregular mural thickenings corresponding diffuse mural oedema (Galvin *et al*., 2004).

4.1.6. Histopathology

Lesions can be both subacute and chronic in nature. In chronic lesion are present remnants of mucosa and sheets of fibrous connective tissue in the lamina propria underlying the ulcerated mucosa and subacute lesions are characterized by a fibrinonecrotic ulcerative colitis (Karcher *et al*., 1990). The lesions were characterized by scaling of the superficial epithelium, necrosis of the lamina propria and degeneration of the capillary walls with associated microthrombosis, extensive neutrophil infiltration, extravasation of red blood cells and edema (Meschter *et al*., 1990). Mucosal ulceration with varying degrees of granulation tissue, oedema and a mixed inflammatory infiltrate (Karcher *et al*., 1990). General, it is characterized by diffuse and superficial epithelial necrosis with scattered erosions and ulcerations (Karcher, *et al*., 1990). Ephitelial necrosis and ulceration, mononuclear cell infiltration into the lamina propria, and marked submucosal edema and fibrosis, with smaller and depleted GALT (Geor *et al*., 1989).

4.1.7. Diagnosis

It is clinically challenging. In general, the recognition of right dorsal Colitis in equines is based on knowledge of its causes, clinical history of the patient, clinical signs and results of diagnostic tests (Cohen, 2002). The differential diagnoses for RDColitis include gastric ulceration, inflammatory, neoplastic or parasitic bowel diseases, and hepatopathies (Cohen *et al*., 1995; Galvin *et al*., 2004).

Santos *et al*., 2009, lists, also, some differential diagnoses when there is evidence of loss of GI proteins, namely: strongilosis, cystostomy, infectious granulomatous enteritis (*mycobacterium tuberculosis, mycobacterium paratuberculosis*, *histoplasma capsulatum*), gastrointestinal neoplasia (such as lymphoma, adenocarcinoma or leiomyosarcoma), granulomatous enteritis, lymphoplasmacytic enteritis or eosinophilic enteritis, congestive heart failure and chronic liver disease.

The diagnosis of right dorsal colitis is based on clinical signs, the history of administration of non-steroidal anti-inflammatory drugs, detection of serum hypoproteinemia and, by ultrasound imaging, necropsy or surgery, the increase in the thickness of this segment of the Large Intestine (Karcher *et al*., 1990; Cohen *et al*., 1995; Jones *et al*., 2003; Galvin *et al*., 2004; Davis, 2017). Furthermore, include a physical examination, haematology, a serum biochemistry profile, urinalysis abdominal ultrasonography (Cohen, 2002; Davis, 2006), abdominocentesis (analysis of peritoneal fluid) and gastroscopy (Galvin *et al*., 2004).

Faecal samples for occult blood research make a potential adjunctive test (Cohen *et al*., 1995; Galvin *et al*., 2004; Andrade *et al.*, 2016; Davis, 2017) but this may be uncertain (as this is neither sensitive nor specific) (MacAllister *et al*., 1993; Cohen *et al*., 1995; Davis, 2006; Mozaffari *et al*., 2010).

Transabdominal ultrasound examination is effective as an early diagnostic aid for diffuse or focal right dorsal colitis if located on the parental faces of the colon, since it has identified thickening of the intestinal wall before significant clinical signs such as diarrhea and dehydration (Andrade *et al.*, 2016) but should be applied and interpreted with full regard for other clinical findings and results of other diagnostic procedures, many which it can complement (Scharner *et al*., 2002).

Direct visualization, such surgical exploration (celiotomy), laparoscopy or necropsy, provides the clinician with a definitive diagnosis and determines the severity of the lesions (Karcher *et al*., 1990; Hough *et al*., 1999; Cohen, 2002; Galvin *et al*., 2004; Davis, 2006; Reed *et al*., 2006; Amaya & Flórez, 2011; El- Ashker *et al*., 2012).

Nuclear scintigraphy also been as a diagnostic tool for Colitis and it can differentiate the severity of colitis based in intensity of WBC uptake (East *et al*., 2000). Other procedures that may be useful in the diagnosis of RDColitis include laparoscopy and exploratory celiotomy, but there are some disadvantages for each of these diagnostic options (Davis, 2006).

4.1.7.1. Ultrasonography

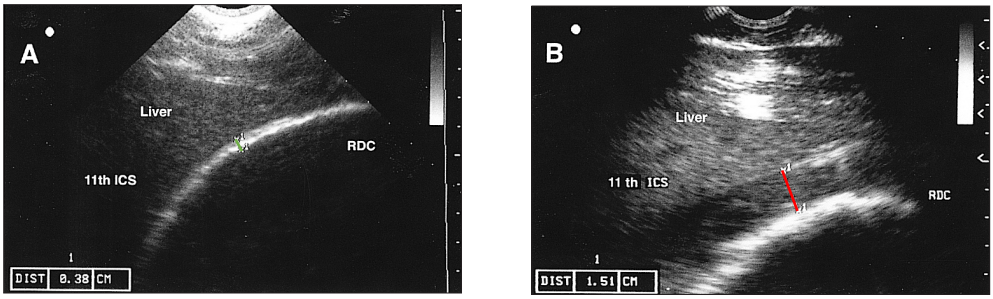
The use of ultrasonography to aid in the diagnosis of RDColitis has also been reported and is widely known among equine practitioners (Cohen *et al*., 1995; Cohen, 2002; Jones *et al*., 2003). To visualize the RDC, the 10th, 11th, 12th, 13th and 14th rights intercostal spaces (ICS) should be scanned, especially the 11th, 12th and 13th that provide more consistent images, where is localized below the margin of the lung and axial to the liver (Jones *et al*., 2003; Galvin *et al*., 2004). The duodenum and the liver will be in the dorsal plane with the ventral colon ventrally (Galvin *et al.*, 2004).

In a study by Siwinska *et al.*, (2017), the RDC of ponies and miniature horses was identified in all the horses in the 12th intercostal space (100%), 96% in the 13th, 87% in the 11th and 70% in the 14th. Hence, it may be assumed that this particular ICS provides the best site for RDC analysis.

The echogenicity presented by an inflamed colon, of course, differs from the echogenicity pattern of a normal colon. In the study by Jones *et al*., (2003), the affected colons presented a thick hypoechogenic layer (smaller than that of the liver), corresponding a submucosal edema, inflammatory cellular infiltrates, and granulation tissue, delimited by the serous and mucosal hyperechogenic layers that interrupted the normal stratification of the bowel layers. The thickness of the hypoechoic layer varied from <50 to> 75% of the total mural thickness (Jones *et al*., 2003).

In the study by Lane *et al*., (2010), the wall of the RDC was heteroechoic with areas of increased echogenicity (relative to the liver) in the submucosal layer, compatible with cellular infiltrate. Freeman, (2002b), in your article, wrote a total wall thickness of greater than 4mm is consistent with abnormal thickening, usually as a result of infiltration with edema, hemorrhage or inflammatory cells.

Figure 11- Ultrasonographic images of the Right Dorsal Colon (RDC) in a healthy horse (A) and a horse with RDColitis (B). (Adapted: Jones *et al*., 2003)



Legend: Cursors indicates the wall of the RDC. There are a marked thickening of the RDC in the horse with RDColitis (red) versus the healthy horse (green). A prominent hypoechoic layer is evident in the wall of the RDC in the affected horse.

Davis, (2017), wrote that colon wall thickness may not increase above normal for up to 72h after initial clinical signs, and this is disapproved by Andrade *et al.*, (2016), where the initial signs appears (apathy, inaptancy, depression, prostration, anorexia), at the same time the increase colon wall thickness.

In a study of Cohen *et al*., (1995), ultrasonography may be useful of diagnosis of stricture, evidencing thickening of the colon wall. Knowing that the diameter of the RDC is physiologically about 50cm (Krunkosky *et al*., 2017), the stenosis described indicates 60% to 70% of the reduced intestinal lumen (Hough *et al*., 1999).

The wall thickness as measured by sonography, of the RDC is higher in horses with RDColitis than in healthy horses and correlates with the actual thickness in the horses in which they were necropsy targets (Jones *et al*., 2003). Another method for confirming RDColitis is to measure the wall thickness of the right ventral colon for comparison and to make the ratio, which will be higher in horses with RDColitis (Jones *et al*., 2003). The thickness of the intestinal wall depends on the content of the intestine and the intensity of the peristaltic contractions (Fleischer *et al*., 1981). It may be assumed that decreasing the contents in the intestines would lead to a thickening of the intestinal wall because it is not distended (Fleischer *et al*., 1981). The large colon appears as a hyperechoic, slightly curvilinear line with sacculations (Scharner *et al*., 2002). Colitis where there are increased fluid content, luminal contents appear as homogenous, hyperechoic material and the colon wall may appear thickened (Scharner *et al*., 2002).

Ultrasonographic wall thickness average values in horses of small breeds (118kg a 380kg) were different from the values obtained for horses of large breeds (498kg a 574kg) (2,7 ± 0,3 mm versus 3,7 ± 0,3 mm, respectively) (Siwinska *et al*., 2017). This suggests that different reference values should be used in small horse breeds when performing an ultrasound examination (Siwinska *et al*., 2017). The mural wall thickness of the RDC in all the ICS was significantly lower ($P<0.001$) in the study group than in the large breed horses from the control group (Siwinska *et al*., 2017).

From the 5th day of treatment there was a significant increase in the mean thickness of the wall of the right dorsal colon, which reached its maximum on the 9th day of treatment with 9,4mm thickness, changes that returned to normal when the experiment ended (Andrade *et al.*, 2016.). Inflammation of the RDC increased the thickness of the wall due to the presence of edema (Andrade *et al.*, 2016). Increased wall thickness is a feature of intestinal strangulation (torsions), enteritis and colitis, inflammatory bowel disease and neoplasia, or peritonitis, or chronic or severe impactions (Freeman, 2002b). Andrade *et al.*, (2016), concludes with his study that the increase in the thickness of the right dorsal colon, synonymous of inflammation, was significantly and negatively correlated with the concentrations of Total Protein and serum

albumin and with the counts of leukocytes and segmental neutrophils. That is, as the intestinal wall increased in thickness, the values of the aforementioned parameters diminished in the blood.

4.1.7.2 Clinicopathological changes.

The most consistent clinicopathological changes features include anemia, hypoproteinemia, hypoalbuminemia and hypocalcemia, probably because to loses through the damaged intestinal mucosa (Karcher *et al*., 1990; Cohen *et al*., 1995; Davis, 2017).

4.1.7.2.1 Hypoproteinemia and hypoalbuminemia

The loss of protein may be due to insufficient intake, insufficient production, increased metabolism or extravascular protein loss (Cohen *et al*., 1995). Hypoproteinemia may occur in some patients even in the absence of changes in the gastrointestinal mucosa as a consequence of inhibition of hepatic protein synthesis, mainly albumin and acute phase proteins, in addition to renal losses (Melo *et al*., 2009).

Hypoproteinaemia is considered when PT values decrease below 40-55g / L and is considered hypoalbuminemia when serum albumin values fall below the reference range of 15-20g / L (Cohen, 2002), but each laboratory has its own reference values.

The mechanism by which NSAIDs induce protein loss and ulceration in RDC is due to inhibition of COX and subsequent loss of mucosal cytoprotective prostaglandins (Lees & Higgins, 1985; Meschter *et al*., 1990), where extensive loss of mucosal barrier, increased permeability explains these changes (Andrade *et al.*, 2016). Horses with RDColitis, therefore, must have leaked significant protein from their visibly ulcerated intestine to the lumen (Karcher *et al*., 1990; Hough *et al*., 1999; Melo *et al*., 2009), and because of the small albumin size, this is preferentially lost in the GI tract in horses with severe intestinal inflammation.

Several studies indicate that occurs hypoproteinemia and hypoalbuminemia (Karcher *et al*., 1990; MacAllister *et al*., 1993; Cohen *et al*., 1995; Hough *et al*., 1999; Jones *et al*., 2003; Galvin *et al*., 2004; Reef *et al*., 2004; Andrade *et al.*, 2016). Hypoalbuminemia is one of the earliest signs of RDColitis, occurring as early as 3 days following commencement of administration of phenylbutazone in experimental studies (McConnico *et al,*. 2008). Hypoproteinemia and hypoalbuminemia may exacerbate hypovolemia (because of decreased intravascular oncotic pressure), further predisposing to intestinal damage induced by NSAIDs (Cohen *et al*., 1995; Cohen, 2002) and note that dehydrated horses may have serum protein values within the reference range (Davis, 2017). Protein losses can result from several pathologies. Enteropathy,

renal disease, diffusion into the abdominal cavity (such as peritonitis) or thoracic cavity (such as pleuropneumonia) may be the sources of decreased serum protein (Davis, 2006). These mechanisms of protein loss may lead to serum protein concentrations below the reference values as 3,5g / dL of total proteins and 1,5g / dL of albumin (Davis, 2006).

4.1.7.2.2 Other abnormal blood findings

Panhypoproteinaemia with hypoglobulinaemia is often in chronic cases or severe disease (Jones *et al*., 2003; Davis, 2017).

Hemoglobin concentration also occurred in phenylbutazone-treated animals (Lees & Higgins, 1987). In most cases the leucograma remains within normal limits, however in some cases a leucocytosis might be observed secondary to significant inflammation. The mean thickness of the RDC was negatively correlated with the mean total leukocyte counts and the mean segmented neutrophil counts, that is, while the mean thickness increased, these hematological parameters decreased in the blood (Andrade *et al*., 2016). Neutrophilia (Hough *et al*., 1999; East *et al*, 2000; Melo *et al.*, 2009) and lymphopenia (Melo *et al.*, 2009) probably resulted from the excess glucocorticoids in the circulation due to stress. Neutrophilia results by the shift of neutrophils from the marginal pool to the circulating pool, decreased neutrophil migration to the tissue and increased mobilization from the reserve pool of bone marrow (Melo *et al*., 2009). Lymphopenia resulted in decreased recirculation or redistribution of lymphocytes within lymphoid tissues (Melo *et al.*, 2009).

Neutropenia with left shift and with toxic changes may also be a finding in the RDColitis when there is systemic impairment of endotoxemia (two horses from the study by Hough *et al*., (1999) evidenced this leukogram). In the large intestine there is an increase in the number of bacteria and endotoxin absorption (McConnico *et al*., 2008), justifying the clinical findings of endotoxemia, the occurrence of phlebitis and laminitis in the study by Andrade *et al*., (2016).

Mechanisms of anaemia in horses with RDColitis have not been determined definitively, but are probably attributable to intra-intestinal blood loss, anemia of chronic inflammation or both (Cohen, 2002). Anaemia is usually mild (Cohen *et al*., 1995; Cohen, 2002; Jones *et al*., 2003) and it had occurred in all NSAID-treatment groups in the study by Mozaffari *et al*., (2010).

Hypocalcaemia may result from reduced dietary intake as result from anorexia as well as reduced protein-bond calcium associated with GI protein loss (Cohen, 2002; Jones *et al*., 2003; Davis, 2006; Melo *et al.*, 2009) and hyperbilirubinemia may be observed and is presumed to result from inappetence or anorexia (Cohen, 2002). There are a few cases of hypomagnesaemia may also have occurred for the same reason (Melo *et al.*, 2009).

4.1.7.3. Necropsy/celiotomy

Many horses suffer euthanasia (and subsequent necropsy) or are subjected to celiotomy (with or without biopsy), which simultaneously confirm the definitive diagnosis of RDColitis through direct visualization (Karcher *et al*., 1990; Cohen *et al*., 1995; Hough *et al*., 1999; Jones *et al*., 2003; Galvin *et al.*, 2004). Concomitantly, acute ulceration of the squamous part of the stomach and mild renal epithelial necrosis may also occur (Hough *et al*., 1999). Postmortem examination of the 15 dead horses showed haemorrhagic and ulcerative inflammation of the entire colon and cecum with varying degrees in all cases (El-Ashker, El-Khodery, Metwally, Hussein & El-Boshy, 2012).

Stenoses/stricture have also been documented in the Karcher *et al*., (1990), Cohen *et al*., (1995), and Hough *et al*., (1999), studies following ulceration. All horses in the study by Hough *et al*., (1999) showed ulcerated dorsal colon stenosis confirmed only by exploratory celiotomy and/or necropsy that indicates 60% to 70% of reduced intestinal lumen (Hough *et al*., 1999).

4.1.8. Therapeutic Management

For resolution of right dorsal colitis, one can choose two types of treatment: medical or surgical (Hough *et al*., 1999; Galvin *et al*., 2004; Davis, 2006). It's more likely the medical treatment provides a more satisfactory outcome if an accurate and early diagnosis can be made and if the owner can be persuaded to comply with the therapeutic and dietary constraints (Galvin *et al*., 2004). In cases of persistent colic pain not responsive to analgesia and marked hypoproteinemia, surgery or euthanasia is advisable (Lane *et al*., 2010).

The principles of medical treatment are based on the discontinuation of NSAIDs, modifying the diet, minimizing stress, the use of prostaglandin therapy (specific medication) (Cohen *et al*., 1995; Cohen, 2002; Jones *et al*., 2003; Galvin *et al*., 2004; Melo *et al*., 2009; Davis, 2017). The decision for surgical treatment occurs when medical treatment fails or when there are chronic complications such as strictures (Cohen *et al*., 1995; Davis, 2017). Decreasing or discontinuing work regimes, avoid any situations that cause stress such changes in management regimes or may precipitate dehydration like long distance transportation is important in the recovery (Cohen *et al*., 1995; Cohen, 2002; Galvin *et al*., 2004). Discontinuation of NSAIDs therapy is mandatory because the healing of ulcerated colonic epithelium may be impaired in the presence of continued prostanoid inhibition (Campbell *et al.*, 2002). Eliminating the cause of the disease is crucial to nullifying the harmful stimulus. If NSAIDs causes RDColitis, so, NSAIDs have being need removed of therapeutic, even when are indicated for treatment of primary disease (such as laminitis, or colic for example). However, being the RDColitis also an inflammatory

condition and it is necessary that an anti-inflammatory is prescribed. Another NSAIDs have need been prescribed that not influence the physiologic COX-1, like COX-2 specific inhibitors, such firocoxib (Davis, 2017). COX-2 specific inhibitors have more safety in respect to the GI tract where doses 12,5 times the label dose administered for 92 days did not result in colonic ulcerations or erosions (Davis, 2017). It has been found that COX-2 also has a maintenance role in relation to the healing process of GI ulcers and that specific inhibition of COX-2 may result in delayed healing (Blikslager *et al*., 1997; Brzozowski *et al*., 2001; Morton *et al*., 2009). Psyllium is a soluble dietary fibre, that gastrointestinal microbes metabolize to form short chain fatty acids (acetate, propionate, butyrate) that help in the healing of the mucosa (Argenzio *et al*., 1974b; Argenzio, 1981; Davis, 2006).

Supplementation with vegetable oil can be an effective and inexpensive option and has been proposed as beneficial (Cohen, 2002). In a study by Cargile, Burrow, Kim, Cohen & Merrit, (2004), corn oil, rich in 60% linoleic acid, an precursor of PGE_2 (Hough *et al*., 1999), was shown to significantly increase the production of PGE_2 and simultaneously decrease gastric acid secretion by gastric cells with an increase in sodium secretion (probably related to bicarbonate of sodium to buffer stomach acid). Therefore, it may be added to the therapy as a protector of the mucosa against ulceration and it improves mucosal healing (Cargile *et al*., 2004). Also, corn oil is an additional source of low-bulk calories for weight loss cases of RDColitis (Davis, 2017) in dose 100 to 200ml daily (Cohen *et al*., 1995; Cargile *et al*., 2004). Safflower oil likewise has recommended as a prophylactic supplement for horses with gastric ulceration (Davis, 2006).

Misosprostol, is a synthetic analogue of prostaglandin E1 and may be part of the medical treatment of RDColitis, in the dose of 2-5µg/kg btw PO every 6-12h (Cohen, 2002; Davis, 2006; Davis, 2017). It can help epithelial healing and protection (Davis, 2006), but cost and side effects like abdominal discomfort limit its use in horses (Galvin *et al*., 2004). This drug cannot be given to pregnant animals because it has abortive properties.

Sucralfate is a cytoprotective agent for treatment of gastric and duodenal ulcers in human (Davis, 2017) and used in humans with colitis (Davis, 2006). It forms a viscous sticky gel, which adheres firmly to the base of the ulcers. If gastric ulceration is present, specific therapy include omeprazole (4mg/Kg/day PO) or ranitidine (6,6mg/Kg/PO TID) (Davis, 2006).

Other treatments should be administered as needed based on the individual case. Crystalloid fluids for horses that exhibiting signals of hypovolemia/dehydration and endotoxemia, colloids or plasma for hypoproteinemia because increased the plasma oncotic pressure and aid the reduction of ventral or peripheral edema and edema of the bowel wall (Galvin *et al*., 2004; Davis, 2006; Davis, 2017).

Surgical treatment involves the resection and bypass of the portion of the affected colon (Davis, 2017).

4.1.9. Prognosis

The prognosis of RDColitis is guarded. When detected, the RDColitis is already in an advanced state (Jones *et al*., 2003). There have been reported cases of death or euthanasia by RDColitis induced by NSAIDs (Hough *et al*., 1999; Jones *et al*., 2003; Andrade *et al.*, 2016; Biscoe *et al*., 2018). In one of the earliest reports of right dorsal colitis, 10 of 11 horses in the study were euthanized because of the severity of clinical signs (Karcher *et al*., 1990). In the study by Jones *et al*., (2003), 4 of 5 horses were euthanized, also. But with appropriate and promptly treatment may have a favourable prognosis (Cohen *et al*., 1995; Galvin *et al*., 2004; Davis, 2006; Melo *et al*, 2009).

Complications such, stricture formation (Karcher *et al*., 1990; Hough *et al*., 1999), which evolves to impaction and subsequent colonic rupture (Karcher *et al*., 1990), has a poor prognosis (Hough *et al*., 1999; Cohen, 2002; Davis, 2017). Colonic rupture, also, occurred when there is a thin area of ulceration in the RDC (Jones *et al*., 2003). In the study by Cohen *et al*., (1995), the fact that 2 horses developed colonic stenosis may indicate that the condition of RDColitis evolves into stenosis.

Probably, the prognosis is influenced by the duration of clinical signs (as it determines whether the process is acute or chronic) (Cohen, 2002) and its depends of the precocity of the definitive diagnosis (often presumptive diagnosis) RDColitis and also on the economic situation and compliance with recommendations for medical management of the owner of the horse (Hough *et al*., 1999; Cohen, 2002). Chronic patients with chronic protein loss and severe diarrhea have a more guarded prognosis (Davis, 2006) and administration of higher doses of NSAIDs associate with more severe changes (Hough *et al*., 1999), and with lower doses also been associate a marked change (Cohen *et al*., 1995). In some horses, the presence of accompanying disease or the development of complications, such as laminitis or diffuse peritonitis, hastened the decision for euthanasia or death (Karcher *et al*., 1990). If the horse survives right dorsal colitis, it should be considered in future administrations that the patient has a greater sensitivity to this type of pharmacological agent (Davis, 2006).

III. Experimental Protocol

1. Objectives

a) To evaluation the thickness of the right dorsal colon in horses by ultrasonography in:

- Health horses;
- Horses under NSAID treatment;
- At T1 (before treatment) and T2 (1 day after end of NSAIDs treatment)

b) To evaluate biochemical changes of serum total proteins and albumin, at:

- T1 (before treatment)
- T2 (1 day after end of NSAIDs treatment)

c) To investigate correlations between thickness of RDC and the following parameters:

- Animal age, gender and breed
- NSAIDs treatment duration and drug used
- Serum total protein and albumin

2. Materials and methods

2.1. Sample Population

All horses appertained to the 4º Esquadrão of Guarda Nacional Republicana (GNR). During the period from 17 September 2018 to 31 May 2019, horses that started a treatment protocol with NSAIDs, independently of the cause of the treatment, were integrated into the project. Dr. Daniela Teixeira randomly chose horses for the study and reasons for NSAID prescription were based on the military surgeon decision, being the majority due to musculoskeletal injuries. Horses admitted and prescribed with NSAIDs were inserted in the experimental **group "B"** while the control **group "A"** was composed of horses without medical records of contact of NSAIDs or at least 2 months free of this type of drugs.

Medical history, gender, age, breed, weight (measured through the thoracic perimeter), iatrotropic stimulus, duration and prescribed treatment were recorded for all horses at admission.

All animals were examined at the time of admission with general physical examination, iatrotropic stimulus record, gender, age, weight (by measuring thorax circumference with the use of a specific tape measure for this function in the equine species). For horses in group "B"

included: prescribed NSAID, administered dose (ml/kg) and duration of treatment. Table 1 shows the individual registration (see Appendice 1).

All horses have similar diet, handling and training. The herd is composed of mares or geldings to avoid behaviours problems attributed to males. Most of the horses in this military unit are Puro Sangue Lusitano (PSL) horses.

Horses are fed 4 times a day (7:30 a.m., 11:30 a.m., 4:30 p.m. and 8:30 p.m.) with hay thirty minutes before each concentrate meal. The body condition of all animals ranges from 3 to 4 (on a scale of 0-5) (Henneke, Potter, Kreider & Yeates, 1983; Carroll & Huntington, 1988), which matches their purpose for competing and performing ceremonies where aesthetics are crucial. In teamwork, grooms daily ensure the maintenance of the beds and their cleaning. Whenever necessary, horses are referred to the hospital, where they can remain in the intense care unit or return to their stable, with daily treatment under veterinary supervision until the end of the treatment.

2.2. Ultrasonographic examination

Ultrasonographic exam at "T1" (first ultrasound record = D1) and "T2" (last ultrasound record (D2). For security reasons, all horses were kept in the stalls during procedures. The transabdominal ultrasonography of the RDC was performed on the first day of treatment (T1), and one day after the treatment was completed (T2). The author of the present study performed all exams, with punctual help from supervisors.

The preparation of the animal included: cleaning of the right flank and the ribs area with warm water and neutral soap to remove particles and loose hair, oily skin, and drying with a paper towel. A copious amount of 70% alcohol and gel allowed good contact between the probe and the skin. An ultrasound machine (LOGIQ V2 ultrasound system of the GE Healthcare) equipped with a convex probe was used with a frequency of 5MHz. All the other parameters were adjusted in each case until a visualization of a good quality image.

The selection of a good quality image was based on some inclusion criteria, which included the identification of organs like liver or duodenum. Indeed, the parietal aspect of the RDC was identified axially and ventral to the liver, ventral to the duodenum and dorsal to the right ventral colon. The RDC was distinguished from the right ventral because it did not present sacculations and the junction between the dorsal and right ventral colons is easy to visualize because it is V-shaped.

The measure of the RDC wall thickness was determinate by the thickening between the intestinal external layer (serosa) and the beginning of the intestinal contents (mucosal interface).

From each animal, 3 ultrasound images were chosen, and the thickness of the colon wall was measured in millimeters in the area where the greatest thickening was detected.

Each individual exam was concluded with several images registrations. All exams were backed up on a DICOM format in an external disk for later appreciation by the Horos program, version 3.3.1.

All the measurements were done by two evaluators (PC, RF) always with an agreement consensus. To obtain a measure of the wall thickness of the RDC, three measurements (in millimeters) were performed and the mean of these measurements was used for statistical analysis. The intercostal space in which the ultrasound examination was performed was recorded. A worktable was created in the *Excel* program (*Office 2019*) where measurements of the wall thickness of the RDC were recorded for each ultrasound exam and each horse.

The figures below illustrate some of the described procedures.

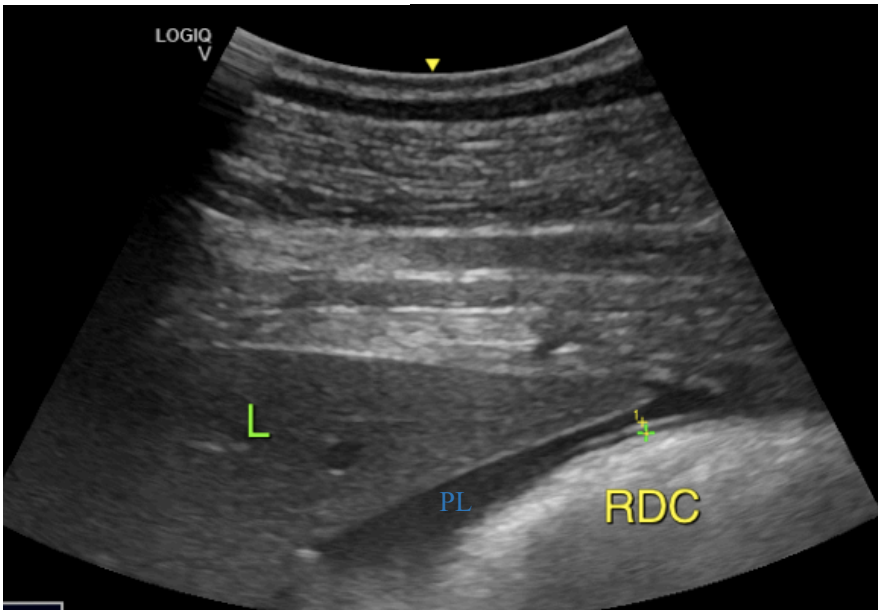
Figure 12- Animal was kept in the stalls during procedure.

Figure 13 and figure 14 refer to one of the animals in the present study, from Group B, a 3-year-old gelding, subject to 3-days NSAID treatment.

At T1, the wall thickness of the ultrasound image of RDC is 1,8mm, an average thickness of 2,2mm. Note the liver, the right dorsal colon, and the hyperechogenic serosa that stands out from the anecogenic peritoneal fluid.

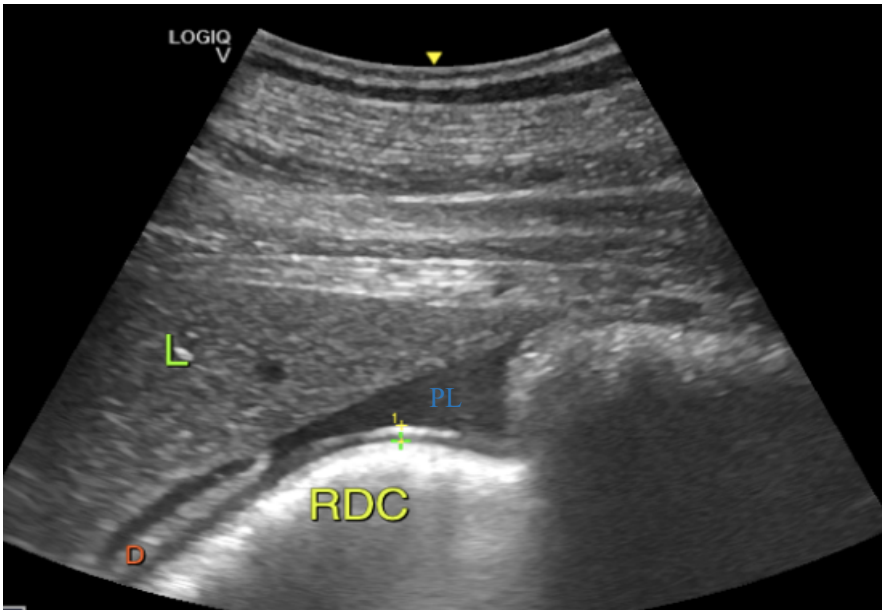
At T2, the wall thickness of the ultrasound image of RDC is 2,9mm. RDC showed a mean thickness of 2,9 mm. The following image is one of three selected ultrasounds. Note the liver, the right dorsal colon, the duodenum and the hyperechogenic serosa that stands out from the anecogenic peritoneal fluid.

Figure 13 - Ultrasound image at T1 (Group B).



Legend: L - Liver; RDC - Right Dorsal Colon; PL- Peritoneal Fluid; 1 - measured in cm from the wall of the Right Dorsal Colon.

Figure 14 - Ultrasound image at T2 (Group B).



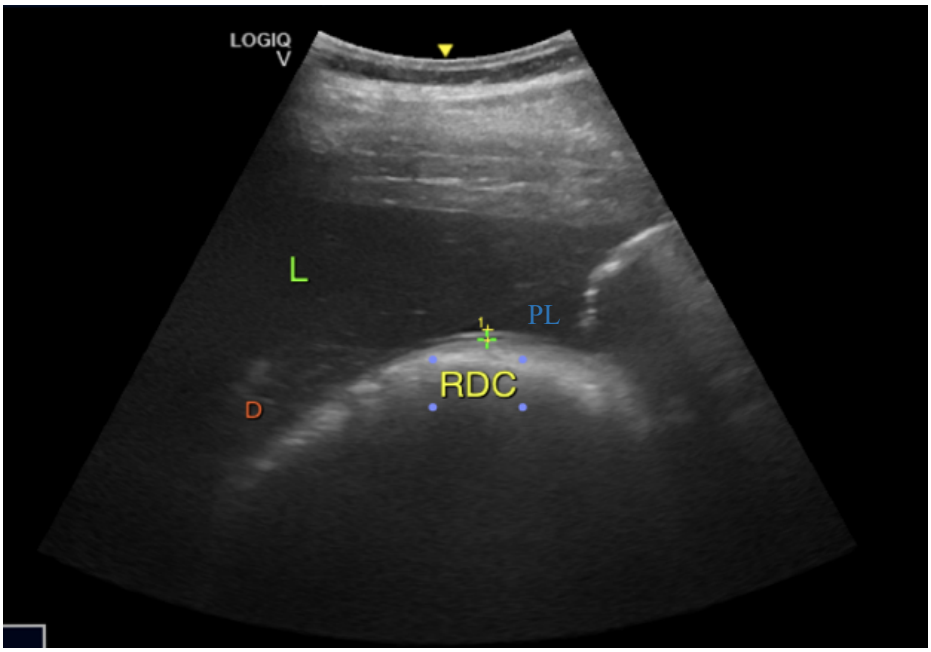
Legend: L - Liver; RDC - Right Dorsal Colon; D - Duodenum; PL- Peritoneal Fluid; 1 - measured in cm from the wall of the Right Dorsal Colon.

The following images (Figure 15 and Figure 16) refer to a 5-year-old female Group A horse, measured at two different times, without influence of NSAIDs.

Ultrasound was performed on the right dorsal colon and showed an RDC wall thickness of 2,5mm, with an average thickness of 2,4 mm. The following image is one of three selected ultrasounds. Note the liver, the right dorsal colon, the duodenum and the hyperechogenic serosa that stands out from the anecogenic peritoneal fluid (Figure 15). On the day after the end of the

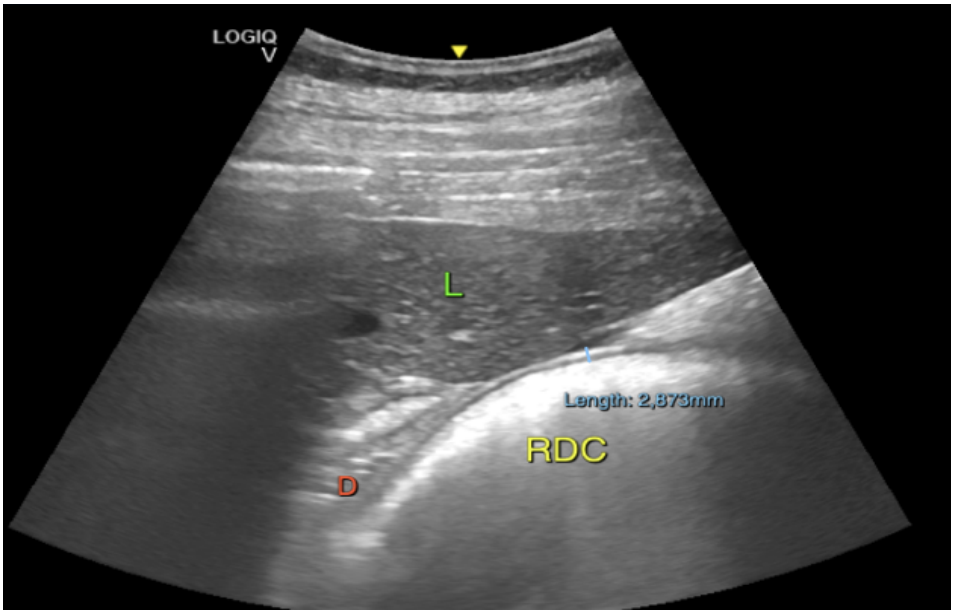
treatment, the right dorsal colon was scanned and showed an RDC wall thickness of 2,9mm, and a average thickness of 2,2 mm. The following ultrasound image is one of three selected ultrasounds. Note the liver, the right dorsal colon, the duodenum and the hyperechogenic serosa.

Figure 15 - Ultrasound image at T1 (Group A).



Legend: L - Liver; RDC - Right Dorsal Colon; D - Duodenum; PL- Peritoneal Fluid; 1 - measured in cm from the wall of the Right Dorsal Colon

Figure 16 - Ultrasound image at T2 (Group A).

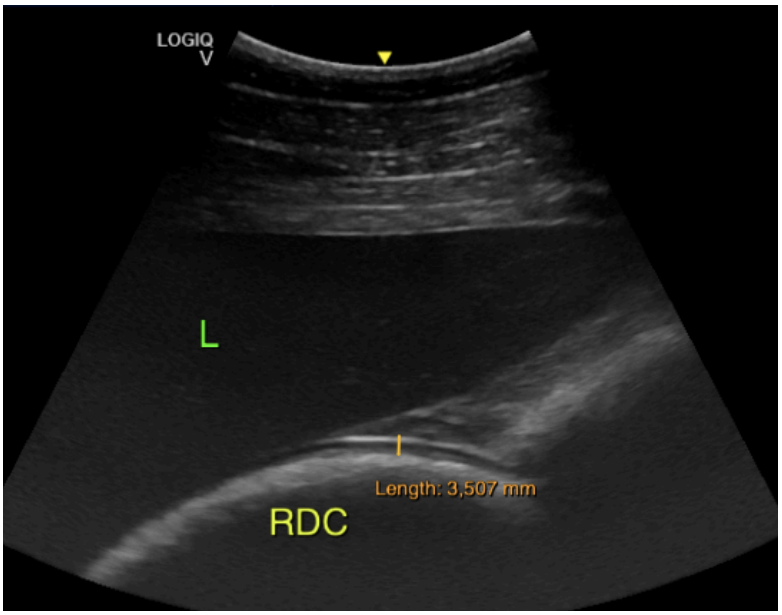


Legend: L - Liver; RDC - Right Dorsal Colon; D - Duodenum; Length - measured in mm from the wall of the Right Dorsal Colon

The following echographic images (figure 17 and figure 18) refer to some of the older horses from Group A.

- Gelding horse with 22 years.

Figure 17 - Ultrasound image at T1 (Group A).

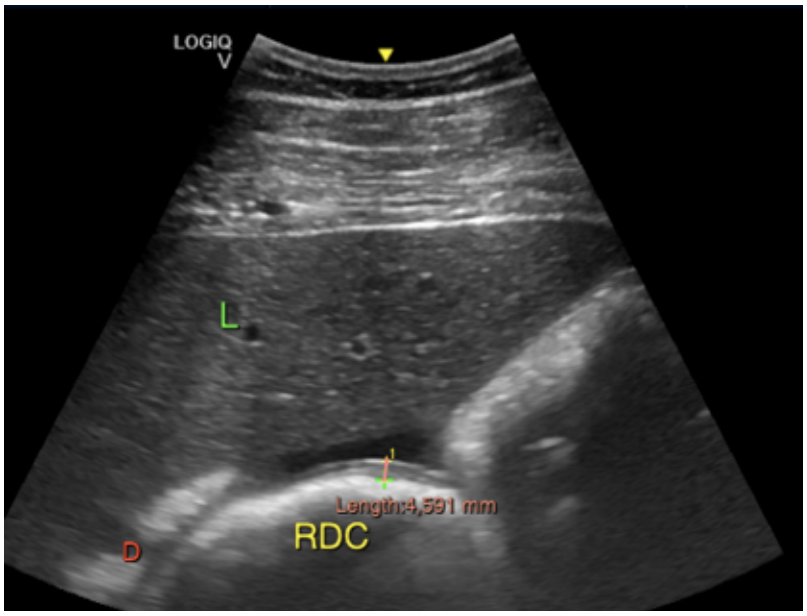


Legend: L - Liver; RDC - Right Dorsal Colon; D: Duodenum; Length - measured in mm from the wall of the Right Dorsal Colon.

Length = 3,507mm

- Gelding horse with 24 years.

Figure 18 - Ultrasound image at T1 (Group A).



Legend: L - Liver; RDC - Right Dorsal Colon; D: Duodenum; Length - measured in mm from the wall of the Right Dorsal Colon.

Length = 4,591mm

2.3. Blood Analysis

On first day of treatment (day 1 – D1) blood samples were collected from each horse. Blood sample were also obtained one day after administration of the last treatment (T2). Cotton soaked in alcohol was used to disinfect the site and the jugular vein (right or left) was punctured for blood sampling. One blood sample was collected in the control group on the day ultrasound was performed (T1). In relation to the experimental group there were two collections of blood at different times:

• T1 corresponding to the day of admission and the day the first dose of nonsteroidal anti-inflammatory was given;

• T2 corresponding to the day after the last day of the non-steroidal anti-inflammatory therapy.

All blood samples were collected in heparin vacuum tubes to determine the concentrations of serum albumin (Alb) and Total Proteins (PT). The needles used were 20G x 1 "(0,9x25mm). This vacuum system is called BD Vacutainer, PrecisionGlide (Multiple Sample Blood Collection Needle). The blood samples were stored in the cold at ± 4 ° C until they were delivered to the analysis laboratory. The laboratory responsible for the analysis of the blood samples was the Laboratory of Clinical Analysis Braço Forte, headquartered at the Faculty of Veterinary Medicine of the University of Lisbon.

2.4. Data analysis

All data collected from the study were entered into a Microsoft Office Excel® database. This database was later exported to the RStudio program, version 1.2.1335, 2009-2019 for statistical analysis.

Based on quantitative variables (mean thickness of the wall of the right dorsal colon), a Kolmogorov-Smirnov test was used to verify the normality distribution of biological variables, as RDC wall thickening (Graph 1).

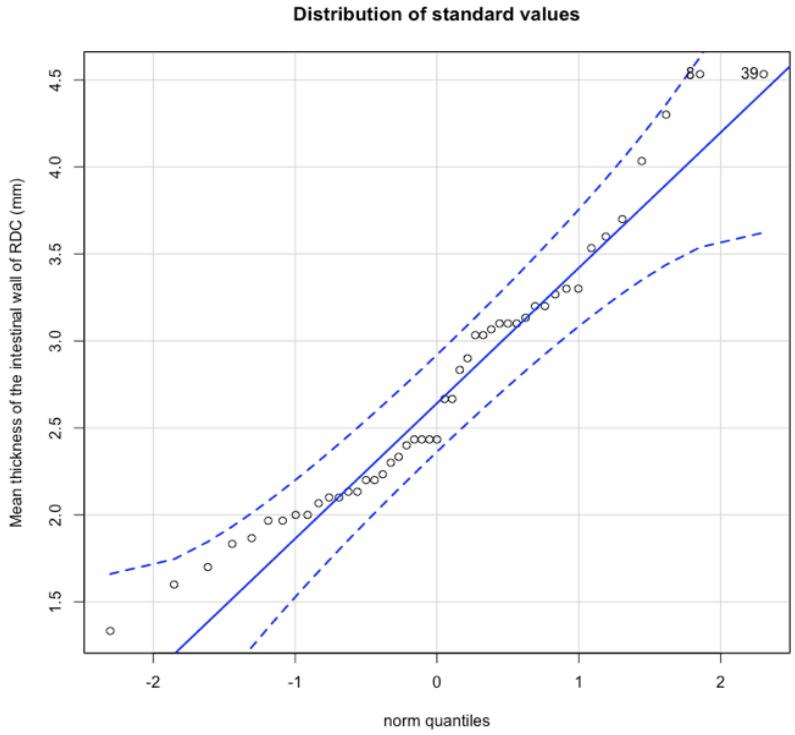
Linear Regression was used to evaluate the relationship between dependent and independent variables, or to investigate the existence of an association between the mean wall thickness (in mm) of the RDC with age and gender of the animals.

An analysis with a mixed model was used with the command of library lme4, where the fixed factors were Age, Group (A or B), Time (T1 and T2) and interaction between these two factors (Group and Time), with Animal being a random factor. Thus, evaluating the effect of NSAIDs on the intestinal wall of the RDC.

In order to relate the variance of the thickness between T1 and T2 with the variance of the PT in the same interval and to relate the variance of the wall thickness of the RDC between T1 and T2 with the variance of the Alb in the same time interval, the Correlation of Spearman's was used. Spearman's correlation was also used to instigate the relationship between the duration of treatment (in days) and the variation of the mean wall thickness of the RDC.

A significance level of 0,05 was used.

Graph 1- Distribution of standard values of the sample this study.



3. Results

3.1 Characterization of the population and measured parameters

A total of 48 PSL horses were included, with 16 mares (33%) and 32 geldings (67%). Group B (Treatment) consisted of 22 animals and group A (Control) of 26 animals. All male horses are neutered for behavioral and management reasons.

Horses aged between 3 and 24 years old, with a mean age of 11,02 ± 6,37 years.

Measures of the wall of the RDC were recorded for group A and group B, with respective registration of the ICS, at T1 and T2. The mean RDC thickness at T1 in group A is 2,83 ± 0,68mm and at T2 is 2,49 ± 0,77mm. In group B, the mean RDC thickness at T1 was 2,53 ± 0,85mm and at T2 it was 2,91 ± 0,7mm. The average thickness of the PSL is 2,70 ± 0,76mm (table 4).

Identification of the RDC was registered between 12th and 14th ICS (table 4).

Table 2 shows the population constitution, in order of gender in group A and group B.

Table 2- Characterization of the study sample, numerical and percentage.

Group / **Gender**	**A** N (%)	**B** N (%)	**Total** N (%)
Male	17 (35,4%)	15 (31,2%)	32 (66,7%)
Female	9 (18,8%)	7 (14,6%)	16 (33,3%)
Total	26 (54,2%)	22 (45,8%)	48 (100%)

Legend: A – Group Control; B – Group Treatment; N - Numerical; % - Percentage.

Table 3 shows the characterization of the population by age (in years).

Table 3- Characterization of the study sample, in age.

Age	Minimum	Maximum	Mean	s.d
(years)	3	24	11,02	6,37

Legend: s.d - standard deviation

Table 4 illustrates the mean measurements of the RDC thickness and the standard deviation, as well as the intercostal spaces that best visualizes the referred organ, according to the group and the different times. Overall, including horses from group A and group B, at T1, the mean of the RDC wall thickening was 2,70 ± 0,76 mm.

Table 4- Mean and standard deviation of the thickness of the Right Dorsal Colon (RDC), in mm, in relation to the group (A or B) and at different times (T1 and T2) and Intercostal Space (ICS) in which the mentioned structure was observed.

		RDC (mm)	s.d.(mm)	ICS
Group A	T1	2,83	0,68	12^{th}; 13^{th}
	T2	2,49	0,77	12^{th}; 13^{th}
Group B	T1	2,53	0,84	13^{th}; 14^{th}
	T2	2,91	0,70	12^{th}; 13^{th};14^{th}
Mean at T1	T1 (A) + T1 (B)	2,70	0,76	

Legend: A – Group Control; B – Group Treatment; RDC - Right Dorsal Colon; mm – millimeters; s.d. - standard deviation; ICS - Intercostal Space.

The next table, table 5, indicates the mean serum albumin and total proteins with their respective standard deviation, at different measurement times and in different groups.

Table 5- Mean and standard deviation of serum Albumin and Total Proteins in relation to group (A or B) and at different times (T1 and T2).

		Alb (g/dl)	s.d. (g/dl)	PT (g/dl)	s.d. (g/dl)
A	T1	3,24	0,31	6,00	0,44
	T2	3,11	0,20	6,07	0,42
B	T1	3,24	0,34	6,49	0,64
	T2	3,23	0,30	6,81	0,57

Legend: A – Group Control; B – Group Treatment; Alb – Serum Albumin; PT – Total Proteins; s.d. - standard deviation; g/dl – grams per deciliter.

3.2 Associations between RDC wall thickening and studied parameters

Only age showed a statistically significant effect on the RDC wall thickening, p-value = 0,0247, as shown in table 6. A total of 48 animals were included in this evaluation. The effect of gender (p-value = 0,5350) was not significant (table 6; graph 2). Additionally, from the linear

regression was possible to stipulate the following relationship between age and RDC wall thickness:

$$y = 2{,}17037 + 0{,}03926x$$

where x = age, in years, and y = mean RDC wall thickness, in mm. This equation stipulates the increased thickness of the RDC with increasing age of the horse.

The Mixed Models were chosen because there are repeated measures over time, that is, the same individual (s) was measured in more than one evaluation condition (time) (table 7). Again, in this analysis, only age has statistically significant (p-value = 0,0041). The relationship between the wall thickness of the initial RDC (T1) and the wall thickness of the RDC at the end of the treatment (T2) was not statistically significant (p-value = 0,399). The relationship between the group (A or B) and the mean wall thickness of the RDC was not statistically significant (p-value = 0,898). Interaction between the Group and Time was also not statistically significant (p-value = 0,114688). For the objective of mixed model analysis, 32 animals (10 females and 22 geldings) were included, 12 animals belonging to group A and 20 animals to group B. Only 32 horses have complete records in T1 and T2. Gender was not included in this statistical analysis because it proved to be statistically non-significant (table 7).

The correlation between the variance of Alb and the thickness of the wall of the RDC, and the variance of the PT with the thickness of the wall of the RDC, with the Spearman Correlation was used (graph 5 and graph 6, respectively). Only horses belonging to group B, at T1 and T2, were used, making a total of 20 animals, 6 females and 14 geldings. The p-value of 0,568 indicates that the Albumin variance was not statistically significant in relation to the mean wall thickness variance of the RDC. The p-value of 0,275 indicates that the variance of the PTs was also not statistically significant with the mean thickness variance of the RDC.

Finally, the relationship between the duration of therapy in days and the mean thickness variation of RDC was analyzed through Spearman's Correlation. The same 20 horses were treated with NSAIDs and recorded at both times (T1 and T2). Table 8 shows all treatments to which group B horses were submitted, i.e., active principle and duration in days. The dose administered took into consideration the animal's weight, following the recommended dosage (4,4mg/ml of PBZ, 1,1mg/Kg FM and 2,2mg/Kg of Ketp). The p-value was 0,049, which indicates a statistically significant influence on the duration of the therapy, in days, on the variation of the mean RDC wall thickness (graph 7).

Then, in Table 6, it can find the linear regression analysis with the factors age, in years, and gender, observing their statistically significant influence on the mean wall thickness of the RDC.

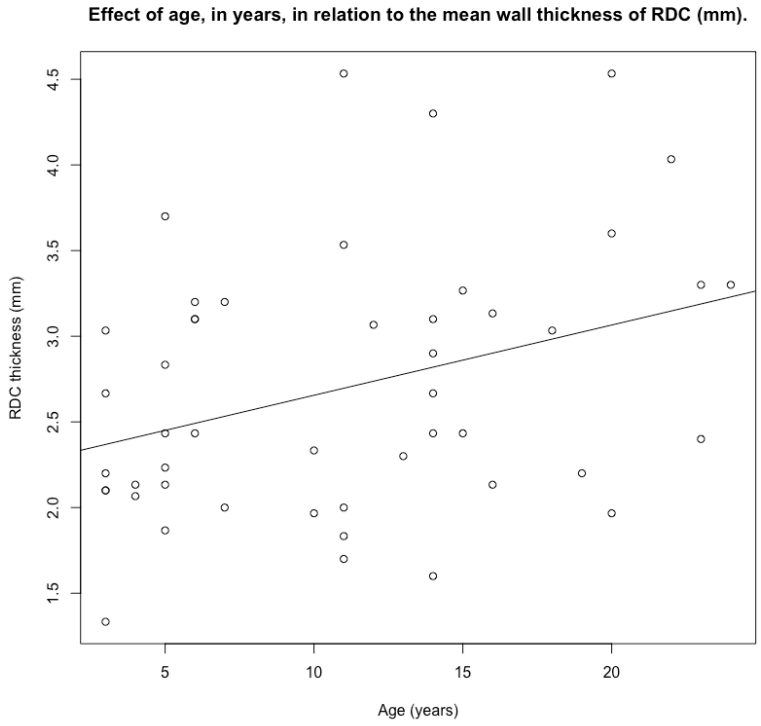
Table 6 - Levels of significance of the factors age, in years, and gender in relation to the mean thickness of the intestinal wall of the RDC.

	Estimate Std.	Error	t value	p-value
Intercept	2,17027	0,24328	8,921	1,64x10^-11***
Age (years)	0,03926	0,01689	2,325	0,0247*
Gender	0,14119	0,22585	0,625	0,5350

Legend: Signif. Codes: * 0,01; *** 0

The graphs below, Graph 2 and Graph 3, illustrate the effect of age in years and gender on the mean wall thickness of the RDC (in mm), respectively.

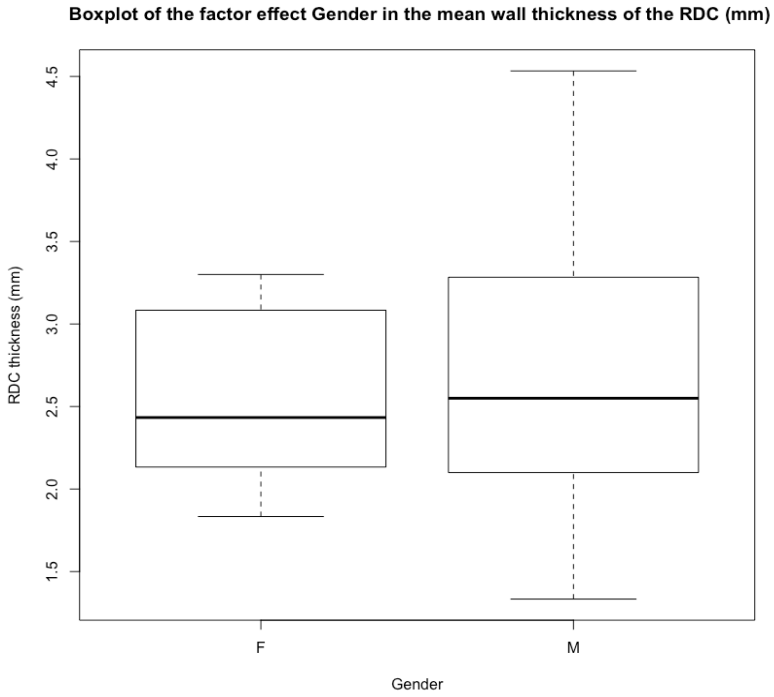
Graph 2- Statistically significant effect of age, in years, on mean wall thickness of RDC (mm)



Legend: RDC – Right Dorsal Colon; mm – millimeters.

$$y = 2{,}17037 + 0{,}03926x$$

Graph 3- Non-statistically significant effect of gender (F or M) on mean wall thickness of RDC (mm)



Legend: RDC – Right Dorsal Colon; F - Female; M – Male; mm - millimeters

Table 7 shows the mixed model analysis. The levels of significance found in the statistical analysis for the mean wall thickness of the RDC are shown in the following table:

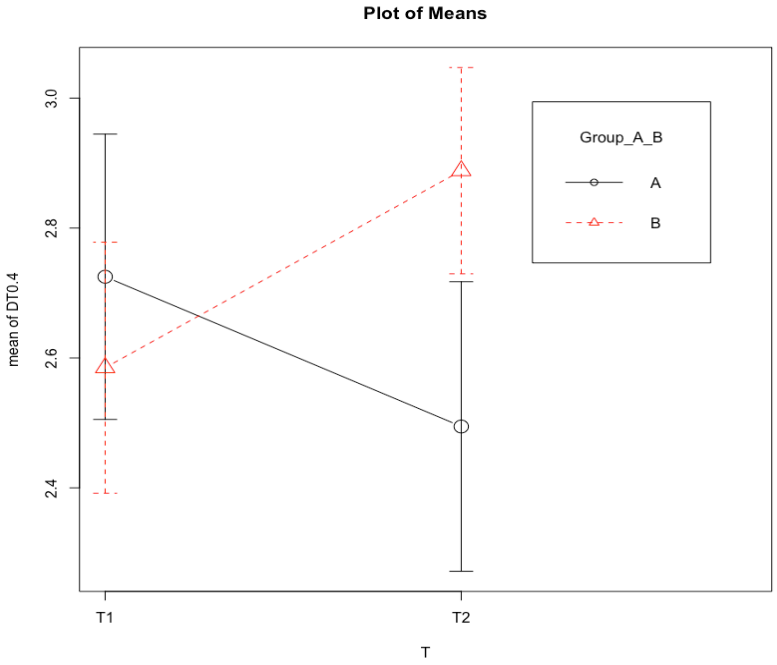
Table 7- Levels of significance of the factors studied (group, time, age, interaction between group and time)

Factor	D.f.	p-value
Group (A or B)	1	0,898151
Time (T1 or T2)	1	0,399034
Age (in years)	1	0,004077**
Interaction between the group and time	1	0,114688

Legend: D.f. – Degrees of freedom; **0,001

Graph 4 shows the influence of time, at T1 and T2, on the RDC thickness mean in both group B (treatment) and group A (control).

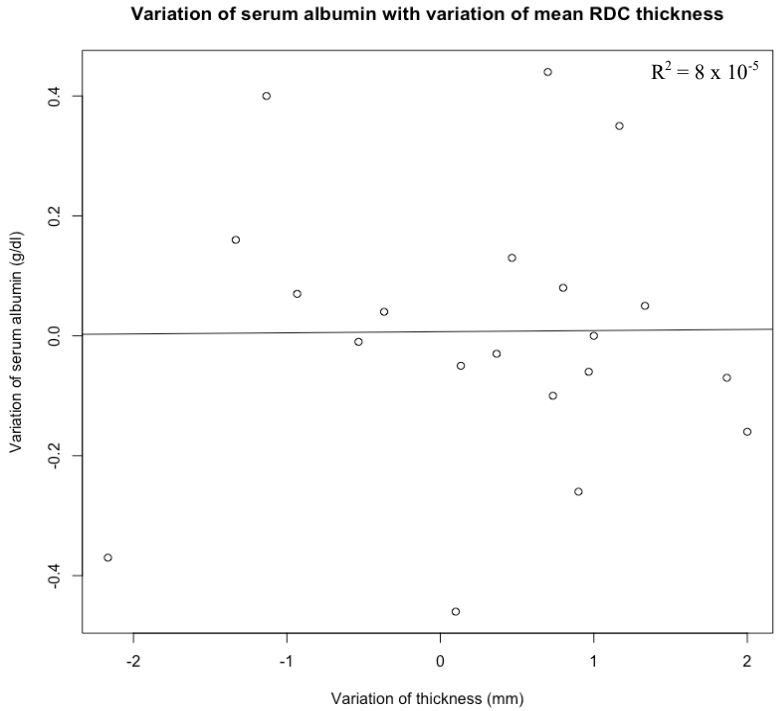
Graph 4- Evolution of the mean wall thickness of the RDC, from T1 to T2, in both groups A and B.



Legend: RDC – Right Dorsal Colon; T – Time; mm – millimeters

Subsequently, the variation of serum albumin with the variation of mean RDC thickness in only group B horses (treatment) was analyzed. Graphs 5 illustrate the aforementioned.

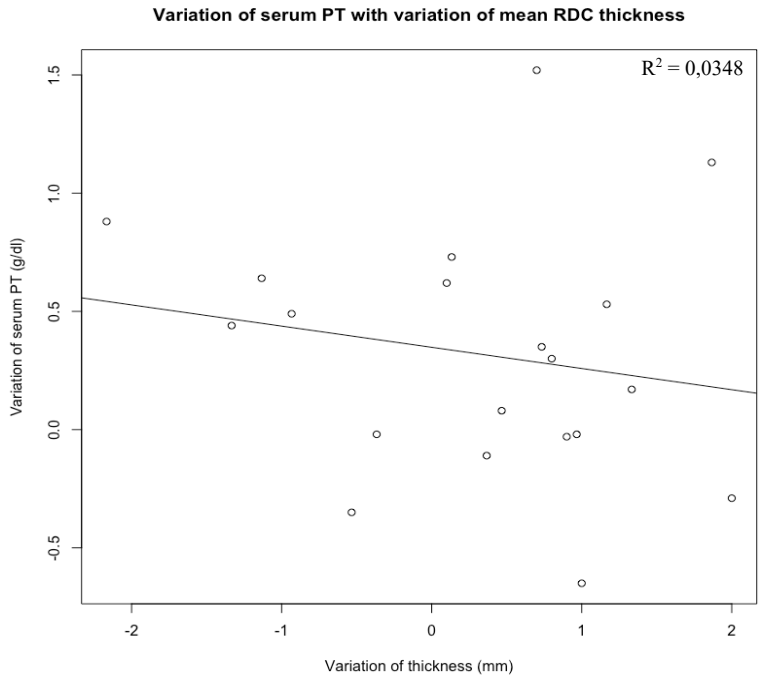
Graph 5- Variance of serum albumin with variation of mean RDC thickness.



Legend: RDC – Right Dorsal Colon; mm – millimeters; g/dl – grams per deciliter

Then, the variation of the total proteins with the variation of the mean thickness of the RDC in only group B horses (treatment) was analyzed. Graphs 6 illustrate the aforementioned.

Graph 6 – Variance of serum PT with variation of mean RDC thickness.



Legend: RDC – Right Dorsal Colon; PT- Total Proteins; mm – millimeters; g/dl – grams per deciliter

Table 8 shows the distribution of treatments in relation to the duration and choice of active principle:

Table 8 – Treatments of group B horses (active principle and duration, in days).

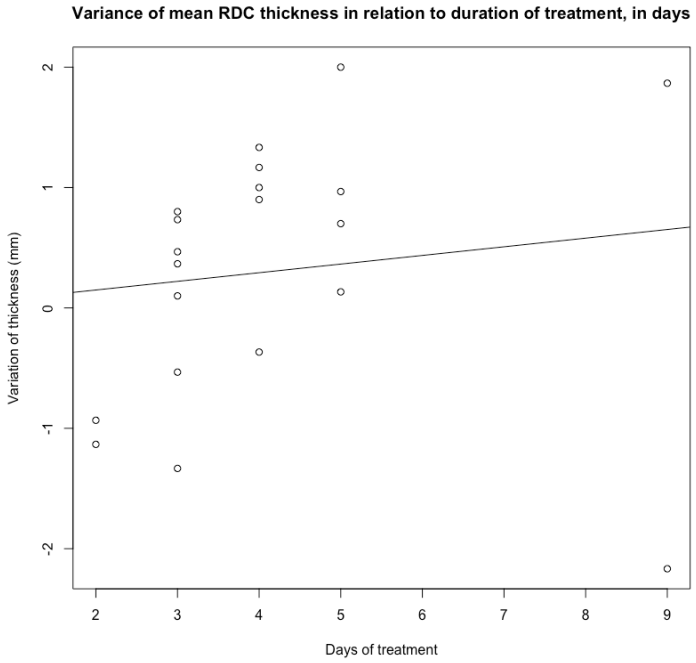
Days / Active principle	2	3	4	5	9	Total
PBZ	2	5	5	4	1	17
FM		1			1	2
Ketp		1				1
Total	2	7	5	4	2	20

Legend: PBZ - Phenylbutazone; FM - Flunixin Meglumine; Ketp – Ketoprofen.

Graph 7 illustrates the relationship between mean RDC thickness and treatment duration in days.

For each day of treatment, the wall thickness of the RDC increases by 0,04458287mm.

Graph 7 – Variance of mean RDC thickness in relation to duration of treatment, in days



Legend: RDC – Right Dorsal Colon; mm – millimeters.

4. Discussion

One of the advantages of the present study is the fact of all animals being subject to the same conditions, as environment, management, feeding, training or stress. Additionally, all horses included in this study population were Puro Sangue Lusitano (PSL), so that results of the study can be referred to the PSL horse breed.

A total of 48 animals were included in the study. The mean wall thickness of the Right Dorsal Colon was measured at two different times (table 4). At T1, the thickness without influence of NSAIDs or other treatment for at least two months is a period recognized for the colon wall to heal (Cohen, 2002; Jones *et al*., 2003; Galvin *et al*., 2004; Davis, 2006). While T2 represents the measurement of RDC thickness on the day following the last day of treatment in group B horses. In total, including group A and B at T1, the average thickness is 2,70 ± 0,76 mm. For a normal average thickness of the RDC, these values are in agreement with other studies (Fleicher *et al*., 1981; Jones *et al*., 2003; Hendrickson *et al*., 2007; Amaral & Froes, 2014). However, Thoroughbred horses have an RDC wall thickness of 3,7 ± 0.3mm (Siwinska *et al*., 2017), showing a higher thickness of the RDC wall when compared with the PSL horses of this study. The RDC was systematically identified at the 12^{th}, 13^{th}, and 14^{th} ICS on the right side, as evidenced in the studies by Jones *et al*., (2003) and Siwinska *et al*., (2017). At the 10^{th} and 11^{th} ICS, visualization of the lung with a reverberation artifact difficulties the identification of the RDC (Siwinska *et al*., 2017), also observed 43% and 13% the presence of the lung, respectively. Moreover, the RDC was visualized in contact with the abdominal wall and also medial to the liver (Amaral & Froes, 2014).

Blood samples were collected on the days of the ultrasound examination at T1 and T2 (Table 5). Based on the laboratory reference values used, there was no detection of hypoalbuminemia or hypoproteinaemia, at neither T1 nor T2. This is later discussed.

The effect of age had a significant influence on, with p-value of 0,0247. This finding means that older PSL horses have a thicker RDC, as shown by the equation y = 2,17037 + 0,03926x (graph 2). Consequently, when a horse is 0 years old, the average wall thickness of the RDC is about 2,2mm, whereas a 25-year-old horse would have a RDC wall thickness of 3,2mm. The gender variable, does not influence the wall thickness of the RDC (p-value=0,5350), which is in agreement with the study of Siwinska *et al*., (2017), where gender also did not show a statistically significant influence on the wall thickness of the RDC (p-value = 0,248). Additionally, table 7 shows that only the age factor in years is statistically significant (p-value = 0,0041).

Analysis of all variables included, as Group (A and B), Time (T1 and T2), Age (in years) and the interaction between group and time showed that there is no significant effect on the mean wall thickness of the RDC on T1 and T2 (table 7), indicating that the effect of NSAID on RDC was not significant. However, when evaluating the effect of age, in conjunction with "group effect" and "time effect" there is still a significant effect of age on the RDC wall thickening (p=0,004077). This can be explained by the inconsistence on therapeutic doses, treatment duration and chosen drug, as also referred on different studies (Hough *et al*., 1999; Melo *et al*., 2009; Amaya & Flórez, 2010; Andrade *et al.*, 2016).

In 1987, Lees and Higgins, studied the clearance of PBZ in ponies. They concluded that clearance on 8 to 10-year-old ponies was slower than on 3-year-old ponies. If slower clearance occurs during treatment with these types of drugs, these animals from older age groups are more susceptible to NSAIDs, showing a longer lasting effect of NSAIDs at RDC level. This may indicate that older horses treated with NSAIDs need special attention even using appropriate dosages. However, this conclusion is with animals being treated, unlike the results of this study, that free of NSAIDs for at least two months, older horses show a thicker wall than younger horses.

One explanation the author proposes for this finding may be all non-steroidal anti-inflammatory therapies that an animal is subjected to throughout life that result in chronic thickening of the intestinal wall of the RDC through a cumulative effect. Furthermore, horses with daily physical activity / training that are predisposed to musculoskeletal disorders and being treated with NSAIDs (Cohen *et al*., 1995; Cohen, 2002; Galvin *et al*., 2004).

There are several reports in human medicine describing colon changes induced by chronic treatment of NSAIDs in older patients. Chronic treatments, during years, with PBZ, diclofenac, and other NSAIDs are described to relieve pain caused by also chronic conditions, as arthritis or polyarthritis, osteoarthritis, rheumatoid arthritis, or degenerative joint disease in patients older than 60 years. In 1969, Bravo and Robert reported that a 70-year-old man with severe arthritis underwent 9-year chronic treatment with PBZ, leading to colonic narrowing (in the sigmoid colon) and ulcers that were microscopically composed of granulation tissue and dense chronic inflammatory infiltrate with 2x3 cm of areas, suggestive of chronic irritation secondary to medication. Earlier, in 1971, Naiken and Rachman reported a case of giant ulceration in the transverse colon of a 72-year-old woman, who had a history of administration of PBZ for pain relief caused by arthritis. Histologically, the colon showed annular ulcers separated by apparently normal mucosa. The length of the ulcers was 5 cm with a circumference of 8,5 cm and the smallest was 1,5 cm with a circumference of 3,4 cm (Naiken & Rachman, 1971).

In 1990, a further justification for the thickening of the bowel wall was published. Cases of severe strictures along the small intestine have been reported histologically consisting of fibrosis bands in the submucosal layer called diaphragm-like stricture, which may cause partial obstruction (extending into the lumen) in patients with chronic NSAID treatment for conditions (Levi *et al.,* 1990). NSAIDs, chronically consumed, were responsible for narrowing the intestinal lumen by depositing fibrosis bands in the submucosal layer. Being physiologically a narrow lumen, which became even narrower, patients showed clinical signs of diarrhea, weight loss, abdominal pain. On ultrasound, these constrictions would lead to noticeable thickening of the intestinal wall. Two years later, a colonic constriction had a luminal diameter of 8mm with apparently normal adjacent mucosa with lumen narrowing and circumferential ulceration (Whitcomb, Martin, Trellis, Evans & Becich, 1992). These authors proposed that both Small Intestine and Large Intestine strictures appear to have a common pathogenesis because both have a similar constitution and reach the same layer levels, in other words, constrictions may occur, as the fibrosis develops and progresses at the site of the persistent central ring ulceration (Whitcomb *et al*., 1992).

Püspök, Kiener and Oberhuber, (2000), reported in men with an average age of 74 years of right colon stenosis associated with prolonged NSAID therapies to treat chronic pain such as polyarthritis or degenerative joint disease. In addition to the characteristic colonic inflammation of this disease, the gross lesion showed ring stenosis with and without ulceration (Püspök *et al.*, 2000). At cross-sectional view, these stenotic areas presented fibrosis and submucosal thickening justified as healing sequelae associated with ulceration (Püspök *et al*., 2000). Also in 2001, a case of colonic stenosis that histologically presented transverse bands in the cecum and ascending colon with fibrotic characteristics of the lamina propria and submucosa, with some ulceration in the center of the constriction is described (Israel, Koea, Stewart, Wright & Frankish, 2001). Another report, state that the formation of strictures results from the healing of these ulcers as submucosal inflammation matures into collagenous scar tissue (Israel *et al*., 2001). While some studies state that gastrointestinal ulcers can heal with a degree of fibrosis causing obstruction (Adebayo & Bjarnason, 2006), others state that it is plausible that cyclic ongoing inflammation and healing can lead to strictures (Zeino, Sisson & Bjarnason, 2010). Thus, this disease called “Diaphragm disease” is related to sustained release preparations of NSAIDs (Geboes, Hertogh & Ector, 2006).

Similarly, in old horses with an intense daily physical exercise, and consequently under NSAIDs treatment, perhaps the effect of these accumulated therapies is reflected in the RDC wall thickening, in older horses. Strictures in the RDC were also found in horses that received high doses of NSAIDs, namely PBZ, in long-term treatments (weeks) and with clinical signs

of weight loss, ventral edema, and colic (Karcher *et al*.1990; Hough *et al*., 1999). The constrictions found in the study by Hough et al., (1999), were accompanied by extensive ulceration and a 60 to 70% reduction in luminal diameter, with a report of a horse having a lumen reduced to 5 cm in diameter. It is natural that these horses presented clinical signs of colic, weight loss and ventral edema due to extravasation of proteins to the intestinal lumen from these types of clinical conditions (Hough *et al*., 1999).

Although the extensive list of reports suggesting a cumulative effect of NSAIDs on the RDC wall thickening, nearly 50% of the horses from our study were free of NSAIDs for at least two months. Moreover, these RDC wall thickening were focal rather than generalized. Moreover, it was difficult to differentiate which layers of the intestinal wall were affected, since the intestinal wall stratification was difficult to visualize. One hypothesis to explain these focal thickening could be the influence of repetitive NSAIDs treatments, over the years, to stimulates the appearance of some strictures in the intestinal wall. Particularly if the time in between NSAIDs treatments is short enough to allow deficient intestinal wall scar recuperation. Indeed, fibrotic changes and submucosal thickening are sequelae associated with NSAID-induced ulceration, gradually.

During wound healing, each phase is an important step on the resolution of the injury, from the inflammatory phase to the fibrotic phase, all play and important role. If phagocytic cells do not eliminate the initial stimulus, for example, damaged tissue from inflammation, fibroblasts that are chemically attracted to the inflammation site through inflammatory mediators are induced (Jones, 2015). These inflammatory stimuli cause fibroblasts to differentiate into myofibroblasts, which results in higher extracellular matrix production (Jones, 2015). Activation of myofibroblasts persists (these are primarily responsible for the local production of extracellular matrix proteins,) and collagen-I rich fibrous tissue is formed, leading to wound contraction (Jones, 2015). Fibrosis leads to the formation of an irreversible scar (with loss of architecture) that impairs organ function (Jones, 2015).

Nevertheless, and as mentioned earlier, the wall thicknesses of the RDC appears focal rather than generalized, ie punctual fibrosis permits the functionality of an organ as a all. Jones, (2015) wrote that the clinical symptoms of fibrotic disease do not appear until it has progressed significantly. According, all horses examined on T1 did not present an abnormal clinical examination, as diarrhea or colic, neither hypoproteinemia or hypoalbuminemia. Thus, it is possible that this focal thickening of the RDC wall did not compromise the intestinal function, and consequently the healthy state of the animal.

Another explanation could be related to the slow inflammatory process and long age of the animal. As horses have a relatively long average life of 25 years and a maximum life of 40

years (McFarlane, Sellon & Gibbs, 2001), an advanced age is also associated with increased production of pro-inflammatory cytokines both in vivo and in vitro (Zeino *et al*., 2010). A study by Adams, Breathnach, Katepalli, Kohler and Horohov, (2008), found that even non-dividing equine T cells are capable of producing inflammatory cytokines, namely IFNγ. In this study, it is further indicated that there are increased productions of IFNγ and TNFα by mononucleated cells from older horses via TCD4 + cells. Older horses have significant increased levels of IL-1b, IL15, Il-18, and TNFα gene expression in the peripheral blood, and serum levels, indicating that there is a disruption of inflammatory cytokines with increasing age. Even thought the mechanism responsible for this process is still unknown (Adams *et al*., 2008). In the present study, maybe some of the factors that promote chronic activation and inflammation of the immune system vary with age. Macrophages are activated by IFNγ and secrete TNFα and IL-1β, which are inflammatory cytokines involved in fibrosis (Jones, 2015). Jones, (2015) writes that high levels of IL-1β are higher under various fibrotic conditions. Both IL-1β and TNFα increase TGFβ1 production and have been implicated in myofibroblast differentiation (Borthwick, Wynn & Fisher, 2013).

On graph 4 is visible the evolution of the RDC mean wall thickness in group A and group B at T1 and T2. Apparently, while the mean wall thickness of RDC of Group A decreases from T1 to T2, the mean wall thickness of RDC of Group B increases at the end of treatment, even if these changes over time are not statistically significant (Table 7). The decrease shown in Graph 4 for Group A animals may be justified by the operator's gained experience overtime (T2), and consequently decrease errors on ultrasound image acquisitions. Moreover the more experience and critical sense, the more easier is to obtain good quality images, and good quality database. These could contributed to an overestimation on the ultrasound measurements. Also the angle of the probe is important in the exact measurement of a structure. 90 ° is the angle the probe has to make with the intended structure (Freeman, 2002a). An inexperienced operator can easily make the mistake of not scanning properly. Because ultrasound is a real-time diagnostic method, bowel movements (contractions) make it difficult to obtain a good quality image, which predisposes to erroneous measurements. Although horses are fed throughout the day and the ultrasound examination adapts to the daily routine of the animal, the presence of content in the colonic lumen influences the thickness of the RDC. Fasting improves ultrasound quality (Pinto *et al.*, 2010) and when the colon is distended it has a lower thickness than when the colon is not distended (Fleischer *et al*., 1981).

In group B, there was an apparent increase in the mean RDC thickness from T1 to T2, but this was not statistically significant. Another justification for a higher T2 wall thickening RDC in Group B animals was maybe justified by the small number of days of treatment. In the study

by Andrade *et al*., (2016), only after the fifth day of treatment, the mean RDC thickness increased significantly. Given that the mode (most repeated value) of the duration, in days, of the present study was 3 days (and then 4 days). Thus it is unlikely to be any significant change in the mean RDC wall thickness (Table 7). It is also important to note that the precision to measures such small measurements (in mm) could most probably difficult such measurements. One of the most characteristic effects of NSAID-induced RDColitis is the loss of serum albumin through the ulcerated and inflamed intestinal wall (Karcher *et al*., 1990; MacAllister *et al*., 1993; Cohen *et al*., 1995; Hough *et al.,* 1999, Jones *et al*., 2003; Galvin *et al*. 2004; Reed *et al*. 2006; Andrade *et al.,* 2016). In the study by Andrade *et al*., (2016), hypoalbuminemia preceded the decrease of PT by two days. If there is a decrease in Alb, PT also decreases by decreasing Alb. McConnico *et al*., (2008), concluded in their experimental study that hypoalminemia is one of the parameters that may occur earlier, that is, three days after the beginning of therapy. It has been reported that there is a negative correlation between RDC intestinal wall thickness and Alb and PT levels in horses undergoing PBZ therapy (Andrade *et al*., 2016). Thus, at the end of the anti-inflammatory treatment, the intestinal wall thickness of the RDC increases and the Alb and PT values decrease. In the present experimental study, in group B, the variation of Alb with variation in the mean wall thickness of the RDC was not statistically significant (p-value = 0.5694), and, the variation of PT with the variation of mean thickness of the intestinal wall of the RDC was not statistically significant (p-value = 0.275). This indicates that, statistically, there was no relationship between the variance of RDC intestinal thickness and the variance of Alb and PT values (graph 5 and graph 6). Obviously, if RDC wall thickness was not affected by the effect of NSAID, in other words NSAID did not cause an increase in intestinal thickness synonymous with inflammation, hypoalbuminemia and hypoproteinemia should not occur.

When looking to the association between the variance of mean RDC thickness and the duration (in days) of NSAID therapy, 20 cases were included. For this analysis, two animals were excluded, because in one case it was not possible to collect blood at T2, and in the other it was not possible to perform abdominal ultrasound at T2. Of these 20 cases, two underwent Flunixin Meglumine (FM), one underwent ketoprofen (Ketp) and seventeen horses underwent PBZ therapy (Table 8). PBZ was the most prescribed anti-inflammatory. The p-value of this correlation was less than 0,05, (p-value = 0,049) indicating that there is a statistically significant influence when correlating the mean thickness variance with the increase in treatment days (graph 7). More indicates that for each day of therapy increase, the average wall thickness of the RDC increases by 0, 04458287mm. The data from this study show that the duration of the treatment factor influences the mean thickness of the RDC. The reviewed literature indicates

that it is the high doses of NSAIDs that cause changes in this specific part of the colon (Galvin *et al*., 2004; Amaya & Flórez, 2011: Andrade *et al*., 2016). However, in the study by Cohen *et al.,* (1995), the doses were adequate, but the duration was excessive, which may eventually meet the results found in this study. In contrast, Andrade *et al.,* (2016), administered high doses of NSAIDs several times a day for a short period compared to the treatment period of up to 30 days in the study by Cohen *et al*., (1995). Results of the study by Andrade *et al.,* (2016), showed thickening and loss of protein by the intestinal tract. Thus, both short high-dose NSAID treatments and long-term doses recommended NSAID treatments influence the wall thickness of the RDC.

5. Study limitations

One of the limitations of the study was the existence of treatments with different NSAIDs. PBZ was the most commonly prescribed active ingredient (given the propensity for musculoskeletal injuries that these sport animals have), making a total of 17 treatments. FM was prescribed twice and Ketp was prescribed once. In total 20 complete NSAID treatment cases were collected.

With a view to further acquisition of NSAID-treated cases, a requirement regarding the duration of therapy was not stipulated, ie there was no minimum number of days allowed for that animal to be included in Group B, which resulted in therapeutic treatments. from 2 days to 9 days. The number of cases was also reduced, the experimental group consisted only of 20 animals that had complete information. The reasons for the few cases collected were: unavailability of the ultrasound (would be at the service of another military unit, 3rd Squadron) for the ultrasound examination to be precisely in T1 and T2; sometimes T1 or T2 culminated at the weekend and it was not possible to move the author of the present study and the veterinarian to the military unit.

The effect of NSAID on the day following the last day of treatment was evaluated by ultrasound and blood sampling, but the effect of NSAID was not evaluated at 1 month, 2 months, 3 months, or long term.

The other parts of the ascending colon of older animals have not been ultrasonographically evaluated to determine whether focal thickening is limited to the right dorsal colon alone.

6. Conclusion

In this study, the measurement of the RDC wall thickness in PSL horses was measured and it was concluded that these animals have an average thickness of 2,70 ± 0,76mm. The intercostal spaces that best visualize the RDC in this race were the 12^{th}, 13^{th} and 14^{th}.

The gender factor does not influence the thickness of the RDC, but the age in years of horses has a statistically significant effect, in other words, the average mural thickness of the RDC in horses increases with age.

The effect of NSAID on the RDC level was not significant, therefore, there were no changes in the biochemical parameters (Alb and PT). Incidentally, the correlation between the variation of the Alb with the variation of the mean wall thickness of the RDC between T1 and T2, and the correlation between the variation of the PT and the variation of the mean wall thickness of the RDC between T1 and T2 was not significant.

A curious result was that the duration of treatment in days was statistically significant, in other words, the most lasting treatments in this study were the ones that most influenced the average mural thickness of the RDC.

PBZ was the most prescribed anti-inflammatory in this study.

IV. Bibliography

Adams, A.A., Breathnach, C.C., Katepalli, M.P., Kohler, K., & Horohov, D.W. (2008). Advanced age in horses affects divisional history of T cells and inflammatory cytokine production. *Mechanisms of Ageing and Development*, 129 (11), 656–664.

Adebayo, D. & Bjarnason, I. (2006). Is non-steroidal anti-inflammaory drug (NSAID) enteropathy clinically more important than NSAID gastropathy? *Postgraduate Medical Journal*, 82(965), 186–191

Amaya, J.M.C. & Flórez, L. M. M. (2011). Intoxicación con fenilbutazona en un equino: reporte de un caso. *Revista CES Medicina Veterinaria y Zootecnia*, 6 (1), 65-71.

Amaral, C.H., & Froes, T.R. (2014). Assessment of equine gastrointestinal tract using transabdominal ultrasonography: a new approach. *Semina: Ciências Agrárias*, 35 (4), 1881-1894.

Andrade, B.S.C., Cassou, F., Aranzales, J.R.M. & Alves, G.E.S. (2016). Efficiency of transabdominal ultrasound examination in aid to early diagnosis of induced right dorsal colitis in horses. *Revista CES Medicina Veterinaria y Zootecnia*, 11(2), 51-60.

Argenzio, R.A., Lowe, J.E., Pickard, D.W., & Stevens, C.E. (1974a). Digesta passage and water exchange in the equine large intestine. *American Journal of Physiology,* 226 (5), 1035–1042.

Argenzio, R.A., Southworth, M. & Stevens, C.E. (1974b). Sites of organic acid production and absorption in the equine gastrointestinal tract. *American Journal of Physiology*, 228 (2), 454–460.

Argenzio, R.A. (1981). Short-Chain Fatty Acids and the Colon. *Digestive Disease and Sciences,* 26 (2), 97-99.

Argenzio, R.A., & Meuten, D. J. (1991). Short-Chain Fatty Acids Induce Reversible Injury of Porcine Colon. *Digestive Diseases and Sciences*, 36 (10), 1459–1468.

Aranzales, J.R.M., Andrade, B.S.C. & Alves, G.E.S. (2014). Orally administered phenylbutazone causes oxidative stress in the equine gastric mucosa. *Journal of Veterinary Pharmacology and Therapeutics*, 38 (3), 257–264.

Barrison, A.F. & Wolfe, M.M. (1999). Management of NSAID-related gastrointestinal mucosal injury. *Inflammopharmacology*, 7 (3), 277–286.

Biscoe, E.W., Whitcomb, M.B., Vaughan, B., Dechant, J.E. & Magdesian, K.G. (2018). Clinical features and outcome in horses with severe large intestinal thickening diagnosed with transabdominal ultrasonography: 25 cases (2003–2010). *Journal of the American Veterinary Medical Association*, 253 (1), 108-116.

Bjarnason, I., Fehilly, B., Smethurst, P., Menzies, I.S. & Levi, A. J. (1991). Importance of local versus systemic effects of non-steroidal anti-inflammatory drugs in increasing small intestinal permeability in man. *Gut*, 32(3), 275–277.

Blikslager, A.T., Roberts, M.C., Rhoads, J.M. & Argenzio, R. A. (1997). Prostaglandins I_2 and E_2 Have a Synergistic Role in Rescuing Epithelial Barrier Function in Porcine Ileum. *J. clin. Invest.,* 100 (8), 1928-1933

Borthwick, L.A., Wynn, T.A., & Fisher, A.J. (2013). Cytokine mediated tissue fibrosis. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1832*(7), 1049–1060.

Bravo, A.J., & Lowman, R.M. (1969). Benign Ulcer of the Sigmoid Colon. *Radiology*, *90*(1), 113–115.

Brzozowski, T., Konturek, P.C., Konturek, S.J., Sliwowski, Z., Pajdo, R., Drozdowicz, D., Ptak, A. & Hahn, E.G. (2001). Classic NSAID and selective cyclooxygenase (COX)-1 and COX-2 inhibitors in healing of chronic gastric ulcers. *Microscopy Research and Technique*, 53(5), 343–353.

Budras K-D., Sack, W.O. & Röck, S., (2011). *Anatomy of the Horse.* (6th ed.). Hannover: Schlütersche

Campbell, N. B., Jones, S. L., & Blikslager, A. T. (2002). The effects of cyclo-oxygenase inhibitors on bile-injured and normal equine colon. *Equine Veterinary Journal*, 34(5), 493–498.

Cargile, J. L., Burrow, J. A., Kim, I., Cohen, N. D. & Merritt, A. M. (2004). Effect of Dietary Corn Oil Supplementation on Equine Gastric Fluid Acid, Sodium, and Prostaglandin E2 Content before and during Pentagastrin Infusion. *America College of Veterinary Internal Medicine,* 18, 545-549.

Carrol, C.L., & Huntington, P.J. (1988). Body Condition Scoring and Weight Estimation of Horses. *Equine Veterinary Journal,* 20 (1), 41-45.

Cohen, N. D., Carter, G. K., Mealey, R. H. & Taylor, T. S. (1995). Medical Management of Right Dorsal Colitis in 5 horses: a retrospective study (1987-1993). Journal of Veterinary Internal Medicine, 9(4), 272—276.

Cohen, N. D. (2002). Right dorsal colitis. *Equine Veterinary Education*, 14(4), 212-219.

Cole, C. (2015). Pharmacology of nonsteroidal anti-inflammatory drugs. In: C. Cole, B. Bentz & L. Maxwell (Eds.) *Equine Pharmacology*. (pp 76-84). Oxford: Wiley Blackwell

Davis, E.G. (2005). Rigth dorsal colitis and dietary management. Proceeding of the NAVC North American Veterinary Conference. *North American Veterinary Conference*, 568–570.

Davis, E. G. (2006). How Do I Diagnose and Manage Right Dorsal Colitis? *Compendium Equine: Continuing Education for Veterinarians*, (Winter), 226–230.

Davis, J. L. (2017). Nonsteroidal anti-inflammatory drug associated right dorsal colitis in the horse. *Equine Veterinary Education*, 29 (2), 104–113.

Davis, J.L. (2018). Pharmacologic Principles. In: S.M. Reed, W.M. Bayly & D.C. Sellon (Eds.), Equine Internal Medicine. (4th ed.). (pp 79-137). Missouri: Elsevier.

East, L.M., Trumble, T.N., Steyn, P.F., Savage, C.J., Dickinson, T.N. & Traub-Dargatz, J.L. (2000). The application of technetium-99m hexamethylpropyleneamine oxime (^{99m}Tc-hmpao) labeled white blood cells for the diagnosis of right dorsal ulcerative colitis in two horses. *Veterinary Radiology & Ultrasound,* 41(4), 360-364

El-Ashker, M., El-Khodery, S., Metwally, N., Hussein, H. & El-Boshy, M. (2012). Prognostic Significance of Oxidative Stress Markers in Colitis Associated with Phenylbutazone Administration in Draft Horses. *Journal of Equine Veterinary Science*, 32(3), 146–152.

Fails, A.D., & Magee, C. (2018). *Anatomy and physiology of farm animal:* Anatomy of the Digestive System. (8th). River Street, Hoboken: Wiley-Blackwell (369-412)

Fleischer, A.C., Muhletaler, C.A. & James, A.E. (1981). Sonographic Assessment of the Bowel Wall. *American Journal of Roentgenology*, 136(5), 887–891.

Frappier, B.L., (2006). Digestive System. In J.A. Eurell & B.L. Frappier (Eds), *Dellmann's Textbook of Veterinary Histology.* (6th ed.). (pp. 170-211). Iowa: Blackwell Publishing

Freeman, S., (2002a). Ultrasonography of the equine abdomen: techniques and normal findings. *Equine Practice,* 204–211.

Freeman, S. (2002b). Ultrasonography of the equine abdomen: findings in the colic patient. *Equine practice,* 262-273

Freeman, S. L. (2003). Diagnostic ultrasonography of the mature equine abdomen. *Equine Veterinary Education*, 15(6), 319–330.

Galvin, N., Dillon, H., & McGovern, F. (2004). Right dorsal colitis in the horse: minireview and reports on three cases in Ireland. *Irish Veterinary Journal*, *57*(8), 467-473.

Geboes, K., Hertogh, G., & Ectors, N. (2006). Drug-induced pathology in the large intestine. *Current Diagnostic Pathology*, *12*(4), 239–247.

Geor, R. J., Petrie, L., Papich, M. G. & Rousseaux, C. (1989). The Protective Effects of Sucralfate and Ranitidine in Foals Experimentally Intoxicated with Phenylbutazone. *Canadian Journal of Veterinary Research*, *53*(2), 231–238.

Griswold, D. E. & Adams, J. L. (1996). Constitutive Cyclooxygenase (COX-1) and Inducible Cyclooxygenase (COX-2): Rationale for Selective Inhibition and Progress to Date. *Medicinal Research Reviews*, 16(2), 181–206.

Gurtler, H., Ketz, H.-A., Kolb, E., Schröder, L. & Seilel, H. (1976). Fisiologia de la digestión y de la absorcion. In: E. Kolb (Eds). *Fisiologia Veterinaria*, (pp 371-375), (2nd). Zaragoza: Editorial Acribia

Gunson, D. E. & Soma, L.R. (1983). Renal Papillary Necrosis in Horses after Phenylbutazone and Water Deprivation, *Vet. Pathol.*, 20, 603–610.

Krunkosky T.M., Jarrett, C.L. & Moore, J.N. (2017). Gross and Microscopic Anatomy of the Equine Gastrointestinal Tract. In: Blikslager, A.T., White II, N.A., Moore, J.N. & Mair, T.S. (eds). *The Equine Acute Abdomen* (3rd) (pp 3-18). River Street, Hoboken: Wiley Blackwell.

Hendrickson, E.H.S., Malone, E.D. & Sage, A.M. (2007). Identification of normal parameters for ultrasonographic examination of the equine large colon and cecum. *The Canadian Veterinary Journal*, 48(3), 289-291.

Henneke, D.R., Potter, G.D., Kreider, J.L., & Yeates, B.F. (1983). Relationship Between Condtion Score, Physical Measurements and Body Fat Percentage in Mares. *Equine Veterinary Journal,* 14 (4), 371-372.

Hough, M.E., Steel, C.M., Bolton, J.R. & Yovich, J.V. (1999). Ulceration and stricture of the right dorsal colon after phenylbutazone administration in four horses. *Australian Veterinary Journal*, 77(12), 785–788.

Israel, L.H., Koea, M.D., Stewart, I. D., Wright, C. L., & Frankish, P. D. (2001). Nonsteroidal Anti-Inflammatory Drug- Induced Strictures of the Colon Report of a Case and Review of the Literature, 1362–1364.

Junqueira, L. C. & Carneiro, J. (2013). O Trato Digestivo. In Histologia Básica (12ª ed.), pp. 279–310. Rio de Janeiro: Guanabara Koogan.

Jones K., (2015). Fibrotic Response to Biomaterials and all Associated Sequence of Fibrosis. In: S.F. Badylak (Eds.). *Host Response to Biomaterials: The Impact of Host Response on Biomaterial*, (189-237) Pittsburgh: Academic Press; 189–237

Jones, S. L., Davis, J. & Rowlingson, K. (2003). Ultrasonographic findings in horses with right dorsal colitis: five cases (2000-2001). *Journal of the American Veterinary Medical Association*, 222(9), 1248-1251.

Karcher, L.F., Dill, S.G., Anderson, W.I. & King, J. M. (1990). Right Dorsal Colitis. *Journal of Veterinary Internal Medicine*, 4(5), 247—253.

Lane, J.K., Cohen, J.M., Zedler, S.T., Hollis, A.R. & Southwood, L.L. (2010). Right Dorsal Colon Resection and Bypass for Treatment of Right Dorsal Colitis in a Horse. *Veterinary Surgery*, 39(7), 879–883.

Lees, P. & Higgins, A. J. (1985). Clinical Pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse. *Equine Veterinary Journal*, 17(2), 83-96.

Lees, P. & Higgins, A. J. (1987). Physiological, biochemical and haematological effects on horses of a phenylbutazone paste. *The Veterinary Record*, 121(3), 56–60.

Levi, B.S., Lacey, G., Price, A.B., Gumpel, M.J., Levi, A.J. & Bjarnason, I. (1990). "Diaphragm-like" strictures of the small bowel in patients treated with non-steroidal anti-inflammatory drugs. *British Journal of Radiology*, *63*(747), 186–189.

MacAllister, C.G., Morgan, S.J., Borne, A.T. & Pollet, R.A. (1993). Comparison of adverse effects of phenylbutazone, flunixin meglumine and ketoprofen in horses. *Journal of the American Veterinary Medical Association,* 202(1), 71-77

Maitho, T.E., Lees, P. & Taylor, J. B. (1986). Absorption and pharmacokinetics of phenylbutazone in Welsh Mountain ponies. *Journal of Veterinary Pharmacology and Therapeutics*, 9(1), 26–39.

Marshall, J. F. & Blikslager, A. T. (2011). The effect of nonsteroidal anti-inflammatory drugs on the equine intestine. *Equine Veterinary Journal*, *43*(SUPPL.39), 140–144.

McCarthy, D. M. (1995). Mechanisms of Mucosal Injury and Healing: the Role of Non-steroidal Anti-inflammatory Drugs. *Scandinavian Journal of Gastroenterology*, 30(Suppl 208), 24–29.

McKenzie III, H.C. (2017). Pathophyiology of Enteritis and Colitis. In: Blikslager, A.T., White II, N.A., Moore, J.N. & Mair, T.S. (eds). *The Equine Acute Abdomen* (3rd) (pp 166-182). River Stret, Hoboken: Wiley Blackwell.

Melo, U.P., Fiório, R.C., Araújo, T.B.S., Ferreira, C. & Santos, P.M. (2009). Intoxicação por fenilbutazona em equino: relato de caso. *Acta Veterinaria Brasilica*, 3(2), 111–116.

Mescher, A.L. (Ed.). (2016). *Junqueira's Basic Histology, Text and Atlas: Digestive Tract.* (14th). McGraw-Hill Education.

Meschter, C.L., Gilbert, M., Krook, L., Maylin, G. & Corradino, R. (1990). The effects of phenylbutazone on the intestinal mucosa of the horse: a morphological, ultrastructural and biochemical study. *Equine Veterinary Journal*, 22(4), 255–263.

McConnico, R.S., Morgan, T.W., Williams, C.C., Hubert, J.D. & Moore, R.M. (2008). Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. *American Journal of Veterinary Research*, 69(11), 1496-1505.

McFarlane, D., Sellon, D.C. & Gibbs, S.A. (2001). Age-related quantitative alterations in lymphocyte subsets and immunoglobulin isotypes in healthy horses. *American Journal of Veterinary Research,* 62(9), 1413-1417.

Morton, A.J., Grosche, A., Rötting, A.K., Matyjaszek, S.A., Blikslager, A.T. & Freeman, D.E. (2009). Expression of cyclooxygenase-1 and -2 in the left dorsal colon after different durations of ischemia and reperfusion in Horses, *American Journal of Veterinary Research*, 70(12), 1536-1544)

Mozaffari, A.A., Derakhshanfar, A., Alinejad, A. & Morovati, M. (2010). A comparative study on the adverse effects of flunixin, ketoprofen and phenylbutazone in miniature donkeys: Haematological, biochemical and pathological findings. *New Zealand Veterinary Journal*, *58*(5), 224–228.

Naiken, V.S. & Rachman, R. (1971). Giant Ulcers of the Transverse Colon. *Journal of the American Medical Association,* 217 (3), 344.

Nasr, M.Y., Fadel, M.S., Noha, A.B., & Elzanaty, A.S. (2014). Studies on abdominal ultrasonography in arabian horses. *Assuit Vet. Med. J.*, 60(143), 9–15.

Noble, G., Edwards, S., Lievaart, J., Pippia, J., Boston, R. & Raidal, S.L. (2012). Pharmacokinetics and Safety of Single and Multiple Oral Doses of Meloxicam in Adult Horses. *Journal Veterinary Internal Medicine,* 26, 1192-1201.

Pinto, P.N.V, Chojniak, R., Cohen, M.P., Yu, L.S., Queiroz-Andrade, M. & Bitencourt, A.G.V. (2011). Comparison of Three Types of Preparations for Abdominal Sonography. *Journal of Clinical Ultrasound,* 39(4), 203–208.

Püspök, A., Kiener, H., & Oberhuber, G. (2000). Clinical, Endoscopic, and Histologic Spectrum of Nonsteroidal Anti- Inflammatory Drug-Induced Lesions in the Colon. *Dis Colon Rectum,* 43, 685-691.

Radi, Z. A. & Khan, N. K. (2006). Effects of cyclooxygenase inhibition on the gastrointestinal tract. *Experimental and Toxicologic Pathology*, 58(2–3), 163–173.

Reed, S.K., Messer IV, N.T., Tessman, R.K. & Keegan, K.G. (2006). Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses. *American Journal of Veterinary Research,* 67(3), 398-402.

Reef, V.B. (Ed.). (1998). *Adult Abdominal Ultrasonography*. Philadelphia: Saudares

Reef, V.B., Whittier, M. & Allam, L.G. (2004). Sonographic evaluation of the adult abdomen. *Clinical Techniques in Equine Practice*, *3*(3), 294–307.

Richter, R-A., Freeman, D.E., Wallig, M., Whittem, T. & Baker, G.J. (2002). In vitro anion transport alterations and apoptosis induced by phenylbutazone in the right dorsal colon of ponies. *American Journal of Veterinary Research*, 63(7), 934–941.

Rötting, A. K., Freeman, D. E., Eurell, J. A. C., Constable, P. D. & Wallig, M. (2003). Effects of acetylcysteine and migration of resident eosinophils in an in vitro model of mucosal injury and restitution in equine RDC. *American Journal of Veterinary Research*, *64*(10), 1205–1212.

Science In 3D. 2008. *The Glass Horse: Equine Colic CD*.

Scharner, D., Rotting, A., Gerlach, K., Rasch, K. & Freeman, D. E. (2002). Ultrasonography of the Abdomen in the Horse with Colic, *1*(3), 118–124.

Sisson, S. (1975). Sistema digestivo do equino. In: R. Getty, S. Sisson & J. D. Grossman, Anatomia de animais domésticos (5th Eds)., pp. 424–465. Rio de Janeiro: W.B. Saudares Company

Siwinska, N., Zak, A., Baron, M., Cylna, M. & Borowicz, H. (2017). Right dorsal colon ultrasonography in normal adult ponies and miniature horses. *PLoS ONE,* 1–12.

Smith, R.S. & Fry, W.R. (2004). Ultrasound instrumentation. *Surgical Clinics of North America*, 84(4), 953–971.

Soma, L.R., Uboh, C.E. & Maylin, G.M. (2011). The use of phenylbutazone in the horse. *Journal of Veterinary Pharmacology and Therapeutics*, 35(1), 1–12.

Tasaka, A.C., (2014). Anti-inflamatórios Não Esteroides. In H.S. Spinosa, S.L. Górniak, & M.M. Bernardi (Eds), *Farmacologia Aplicada á Medicina Veterinária*, (pp 245-260) (5thed.). Rio de Janeiro: Guanabara Koogan.

Tobin, T., Chay, S., Kamerling, S., Woods, W.E., Weckman, T.J., Blake, J.W. & Lees, P. (1986). Phenylbutazone in the horse: a review. *Journal of Veterinary Pharmacology and Therapeutics*, 9(1), 1–25.

Tomlinson, J. & Blikslager, A. (2003). Role of nonsteroidal anti-inflammatory drugs in gastrointestinal tract injury and repair. *Journal of the American Veterinary Medical Association*, 222(7), 946–951.

Wallace, J.L. (1999). Distribution and Expression of Cyclooxygenase (COX) Isoenzyme, Their Physiological Roles, and the Categorization of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). *The American Journal of Medicine,* 107, (6A), 11S-17S

Whitcomb, D. C., Martin, S. P., Trellis, D. R., Evans, B. A., & Becich, M. J. (1992). 'Diaphragmlike' Stricture and Ulcer of the Colon During Diclofenac Treatment. *Archives of Internal Medicine*, *152*(11), 2341–2343

Williams, S., Cooper, J.D. & Freeman, S.L. (2014). Evaluation of normal findings using a detailed and focused technique for transcutaneous abdominal ultrasonography in the horse. *BMC Veterinary Research*, 10(Suppl 1), S5.

Yildiz, H., Yildiz, B., Arslan, K. & Özgür, C. (2001). A mathematical model of the ascending colon of the horse. *Annals of Anatomy,*183, 551-554

Zeino, Z., Sisson, G., & Bjarnason, I. (2010). Adverse effects of drugs on small intestine and colon. *Best Practice and Research: Clinical Gastroenterology*, *24*(2), 133–141.

V. Appendices

Table 1- Admission Form and Treatment Follow-up.

Nome: Calisto	D1	D2	D3	D4	D5
Grupo: C					
Data	**21/10/18**		**23/10/18**		**25/10/18**
Raça: Lusitano					
Género: Masculino					
Idade (anos): 11					
Alimentaçao (tipo, quantidade, frequencia)	**Forragem á descrição, concetrado 3xdia, água á descrição**				
Atitude	**N (normal)**				
FC	**42**				
FR	**12**				
Mucosas	**R (rosadas)**				
Pulso digital	**N**				
Perímetro Toracico (cm)	**179**				
Peso (Kg)	**420**				
AINE	**Fenilbutazona**	**Fenilbutazona**	**Fenilbutazona**	**Fenilbutazona**	
Dose AINE	**10ml**	**10ml**	**10ml**	**10ml**	
Duração do tratamento (dias): 4					
Ultrassonografia	**S (Sim)**		**S**		
Espaço Intercostal	**13, 14, 15**		**13, 14**		**13, 14**
Notas: Castração	T1 **Colheita de sangue**				T2 **Colheita de sang**